The
PATHOLOGY
of
NUTRITIONAL DISEASE

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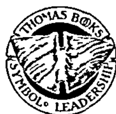
Physiological and Morphological Changes Which Result from Deficiencies of the Essential Elements, Amino Acids, Vitamins, and Fatty Acids

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By

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For

Bunny

Angela and Louise

Preface

"There is perhaps no other subject in medicine where so many contradictory and inexact statements were made, which instead of advancing the research retarded it by leading investigators in a wrong direction." Funk, 1912 (1)

The above statement was made by Casimir Funk over thirty years ago. Unfortunately, what he said then still holds true today. Currently there is much need for a reevaluation of the clinical aspects of nutritional deficiency states, as Dann and Darby have recently pointed out (2). Just as much, however, the physiological and morphological changes which accompany deficiencies in one or more of the essential elements, vitamins, amino acids, and fatty acids require careful and critical analysis, especially with respect to man in whom data which have been accumulated during the past forty years are based on dietaries and observations which are too inadequate when held up to present day standards.

This book has been *formulated to gather together* the available information which deals with the physiological and morphological changes, occurring naturally or produced experimentally, which accompany deficiencies of one or more of the forty odd nutrients now known to be essential. It is hoped that this volume will be of value and a stimulus not only to the pathologist but to workers in nutrition, biochemistry and other fields as well. The importance of the correlation of anatomical and biochemical changes in tissues is obvious enough but is something too infrequently realized in practice. Biochemists can thank the pathologist and histologist for many of the leads which have opened up new horizons in physiological chemistry; the reverse is true as well. But it is unfortunate that the twain so seldom meet.

At the outset some explanation of the scope of this volume must be offered. The field of nutritional deficiencies has become a large one and continues to grow. We have, therefore, purposely restricted the discussion to changes which have been reported in Mammalia. The effects of deficiencies on bacteria can be and have been adequately treated by others. So too, consequences of a lack of one or more essential nutrients on invertebrates and the lower vertebrates, though of great interest to the comparative pathologist, would only confuse any discussion in the present volume. I may be criticized for failure to include tissue changes which have been described in birds. Since, in general, the metabolism of Aves differs greatly from the Mammalia and since some of the tissue changes already described in the two

nutrients are treated For purposes of orientation a brief historical introduction begins each section Such an introduction can of necessity cover only a few of the highlights of the subject for instance as it has been presented the discovery of a certain vitamin would seem to have been a relatively simple matter Such obviously was not usually the case The few papers cited are but a fraction of many reports of investigations carried out before the final solution of a particular problem was reached So too I must ask indulgence of the biochemist and nutritionist who read the various sections which deal with a few of the biochemical relationships of a particular nutrient this section has been added to help orient those who have not kept up with the many advances in this field during the past decade and is not meant to be an exhaustive summary of the subject In the third part of each section Pathological Effects we have tried to include both the anatomical and physiological changes which accompany deficiencies of the various nutrients and have attempted to present such material as clearly critically and precisely as possible and to illustrate the pathological changes whenever this could be done The sources of the material presented in this volume are derived from a review of an appropriate but certainly inadequate literature augmented in a number of instances by histological preparations from our own experiments published and unpublished as many of the illustrations as possible having been selected from such experimental material with which we were familiar

The bibliography which is a condensation of the publications which have been consulted lists most of the now-classical papers especially those dealing with morphological studies Obviously those references to the chemical and physiological roles of the nutrients which are enumerated are only a fraction of the total available This book was not designed to furnish anything but a brief introduction and review of this phase of the subject

A preface does more than attempt to state the purpose and plan of a book it affords the author an opportunity to thank those who have aided in making such an undertaking possible

Numerous investigators have been most generous in allowing me to reproduce illustrations published and unpublished from their work I express my sincere appreciation to Dr S Burt Wolbach Dr J R M Innes, Dr Maurice Sullivan Dr Paul Boyle, Dr Josef Warkany, Dr D T Smith Dr D W Woolley Dr W Buschke and Dr Stefan Ansbacher To the editors of the publications cited in the legends many thanks Others, Dr Karl E Mason Dr Philip Handler Dr E Lowenhaupt Dr D M Greenberg and Dr Maurice Sullivan have kindly allowed me to study and reproduce some of their experimental material

Many of the photographs particularly those of our own preparations were made by Mr Carl Bishop whom I take this opportunity to thank

are so divergent, a discussion of birds has been excluded in order to give the general presentation a little more continuity if such is possible

In the preparation of the various sections of this book the work dealing with experimental deficiencies of single nutrients in animals has been fairly easy to interpret Much of the older literature had, of course, to be omitted since multiple deficiencies were unknowingly being studied It is only when consideration must be given to nutritional disease in man that difficulties arise To many it may come as a shock, therefore, that so little of the total space is devoted to physiological and morphological changes in the human We have tried to bring some order out of the voluminous literature albeit in vain One must realize however that of the forty odd nutrients which are considered in this book only a relatively small number have been shown by themselves to lead to deficiency disease in man except under stringent laboratory conditions Among the clinical syndromes ascribed to nutritional deficiencies some are occasionally uncomplicated scurvy the anemia of iron deficiency possibly the nyctalopia and ocular manifestations of vitamin A deficiency and the hypoprothrombinemia of vitamin K deficiency Among others rickets (alone or in combination with scurvy) beriberi pellagra and even possibly colloid goiter all provide evidence that multiple deficiencies are responsible for the morphological change which are observed In going over the literature dealing with pellagra and beriberi many possible factors are encountered, in the former syndrome one may deal with a deficiency of nicotinic acid riboflavin pyridoxine, iron folic acid thiamine and the quantity or quality of protein, the amount of water ingested sunlight and doubtless countless other factors also seem to play a role

In the pages to follow therefore we have attempted to present those pathological both physiological and morphological changes in man which can be ascribed with some certainty to the deficient factor which is being discussed and have attempted to steer clear of as many of the controversial points as possible especially when we have convinced ourselves that the controversy is staged mainly on a lack of data On the subject of human nutritional disease especially in this country I do not wish to be regarded as a nutritional nihilist However as one who continually observes disease at the autopsy table it is not possible—except in the case of scurvy and rickets in infants—to be other than conservative a leaning which perhaps has some virtue in these days of vitamin inflation

Since this volume has been designed to take up the physiological and morphological changes produced by single deficiencies we have given meager space to the clinical aspects and diagnosis of nutritional disease in the human It is hoped that this treatment will serve to point out more forcibly the defects which are so widespread in our knowledge

Some comment is necessary for the way in which the various essential

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In the preparation of the manuscript Miss Doris Tew has rendered invaluable assistance

To my Chief, Dr Wiley D Forbus, may I express my gratitude for his interest and for making available to me the facilities of this department I should also like to thank Dr Philip Handler for answering many, many questions concerning points dealing with biochemistry

My relations with Mr Charles C Thomas and Mr Payne Thomas have been most cordial and it is a real pleasure to express my appreciation for their keen interest and help in this undertaking

Lastly, I should like to express my respects and appreciation to those with whom I have collaborated To Dr E A Park I owe a tremendous debt for introducing me to the study of nutritional disease by way of the effects of vitamin D and ascorbic acid deficiencies on bone Dr E V McCollum and our fellow collaborators, Drs Harry G Day and Elsa Orent-Keiles taught me a great deal, particularly about deficiencies dealing with the various inorganic elements My associations with Dr M M Wintrobe and his collaborators have been profitable and enjoyable during the period in which we studied the effects of various vitamin deficiencies on swine Finally I extend my heartfelt thanks to Arnold R Rich for his continued stimulation and constructive criticism during the progress of many of the experiments described herein and above all for instilling in this student of disease some of the principles and philosophy of scientific investigation

R H F Jr

Durham, N C
September 1946

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Part I

DIETARY DEFICIENCIES IN GENERAL

"The steady progress in understanding of the biochemistries of the vitamins now obtainable in a pure form is a challenge to the cytologist because in some instances it should be possible to determine the loci, within cells of vitamin activities The opportunity of associating chemical activities or functional roles within nuclear or cytoplasmic structures appears to be at hand'
Wolbach 1937 (3)

PART I

DIETARY DEFICIENCIES IN GENERAL

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PART I

DIETARY DEFICIENCIES IN GENERAL

The preceding quotation can be applied to not only the vitamins, but the essential inorganic elements, amino acids and fatty acids as well. At the present time there is evidence in one or more species for the indispensability of seventeen or eighteen of the ninety odd elements, about fifteen vitamins, ten of the twenty odd amino acids and three closely related unsaturated fatty acids. The term 'indispensability' is based on a number of criteria: growth, maintenance of nitrogen balance and weight, absence of metabolic defect and/or morphological change, normal reproduction, et cetera. Since the pioneer studies of F. G. Hopkins growth has been the criterion most commonly used to determine the dispensability or indispensability of a given nutrient. Normal growth, of course, imposes the greatest possible metabolic drain on the organism and hence brings out most dramatically changes which might only appear slowly or even not at all in the adult organism. Rickets and scurvy are cases in point; though other factors must be considered, these two diseases manifest themselves most flagrantly in the young child during that period when bone growth is so rapid.

Although the pathology of rickets in humans had been described by Pommer (345) in 1885 and changes associated with the scorbutic state by Barlow (468) two years before, the first two decades of this century furnished little else to an understanding of tissue changes which accompany other nutritional deficiencies. It seems fitting to recall the often quoted statement in the British Medical Research Council's *Report on the present state of knowledge of accessory food factors (vitamins)*: 'Disease is so generally associated with positive agents—the parasite, the toxin, the *materies morbi*—that the thought of the pathologist turns naturally to such positive associations and seems to believe with difficulty in causation prefixed by a minus sign" (4). Pathologists of those decades must not be too severely criticised for their failure to study the effects of nutritional deficiencies, inasmuch as the science of nutrition was only then in its infancy. It is true, however, that most of our knowledge of the pathological changes associated with a lack of the essential nutrients has only been accumulated during the past twenty-five years since the above statement was published. But even now the surface has just been scratched and as Wolbach indicated in the opening quotation, there is much to be learned.

Pertinent advances in the elucidation of those lesions which may be pro-

duced by a deficiency of one or more indispensable nutrients in tissues have been made by a relative small group of investigators Wolbach and his co-workers, Howe, Boyle and Bessey have made outstanding contributions the pathology of scurvy and the formation of intercellular substances, epithelial and osseous changes in vitamin A deficiency, dental changes, rickets and lesions resulting from deficiencies of the vitamin B group The publications from Pappenheimer s laboratory are also significant low phosphorus rickets, muscular dystrophy, vitamin E deficiency in a variety of species The Johns Hopkins workers, including McCollum Park Shipley, and their collaborators, and more recently Wintrobe and Sullivan, have aided in elucidating many of the structural manifestations of deficiency diseases rickets, scurvy, deficiencies of the elements, vitamin deficiencies in swine, nutritional dermatoses in rats The important work of certain other investigators will soon come to light in the pages which follow Evans Gyorgy Goldblatt, Mason Lillie Daft, Sebrell and, in England, Mellanby and Innes Those who have contributed to our knowledge of biochemical changes, including growth, must not be lost sight of in this brief resume Such investigators include the group at Yale Chittenden Underhill Osborne and Mendel, workers at the University of Wisconsin, Hart Steenbock Elvehjem and their associates as well as many others Rose Greenberg Woolley and DuVigneaud

A study of the effects of deficiencies of essential nutrients offers a new and appealing approach to the reactions of many tissues to certain injurious stimuli Advances which have proved fruitful have been made in several fields of biological science Some of these should be mentioned and paths for further investigation indicated

For the *histologist* and *histochemist* much has been gained by applying the technique of alterations in tissues produced by deficiency states The controlled formation of intercellular substances in the study of collagen and bone deposition is an eminent example (463) Studies on erythropoiesis have proved and should continue to be fruitful, inasmuch as there are certain essential nutrients which specifically control red blood cell and hemoglobin formation Then too, more precise information on the function and maintenance of myelin might be obtained by studying deficiencies in pyridoxine pantothenic acid (611) and copper (145)

For the *embryologist* investigations of the effects of maternal diets on the off-spring have already yielded important data The relation of riboflavin deficiency to congenital malformations will be discussed in more detail later (5 565) The effects of other nutritional deficiencies on the embryo have hardly been examined Changes associated with a deficiency of alpha tocopherol (402) should stimulate the study of various vitamin and amino

acid deprivations This is a field in human nutrition which is just beginning to be explored (6)

For the *endocrinologist* there are again many problems to investigate The relationship of certain vitamins to degeneration of the liver with consequent effects on estrogen metabolism could be mentioned as an example of what has already been accomplished (550) Investigation of the cytological characteristics of the ductless glands in various nutritional deficiencies should be productive The effects of injections of various hormones in specific deficiencies should yield valuable information on the relationship of hormones and vitamins For instance in this connection it is of interest that when estrogenic hormone is administered to rats, atrophy of the epidermis and sebaceous glands occurs which is very reminiscent of the alterations produced by *riboflavin deficiency* This may be related to the failure of the riboflavin deficient liver to inactive estrogens (550)

The *microbiologist* has only just begun to study the reactions of deficient organisms to bacteria, viruses fungi protozoa et cetera To be sure certain general effects of malnutrition on resistance to infection have been observed, such as depression of antibody formation and phagocytic activity (8), that the former is related to protein reserve has also been shown (9) The effects of deficiencies of all specific nutrients on various animate agents of disease can be investigated with profit For example, it is likely that the intracellular environment of the host may be changed, the course of a subsequent infection with an intracellular parasite such as a virus can then be studied The reactions of mice deficient in various vitamins to injections with the Lansing strain of poliomyelitis and Theiler's encephalomyelitis have been most interesting, the variations in susceptibility are summarized in the following table

Table I

Type of Deficiency	Lansing Strain	Theiler s Strain
Thiamine (10)	+	+
Pantothenic acid (11)	0	+
Riboflavin (12)	±	0
Pyridoxine (13)	0	0
Biotin (13)	0	0
Inositol (13)	0	0

Nutritional deficiency disease as an adjunct to cancer research has already opened new approaches for the *oncologist* The relationships of biotin and pyridoxine to the production of liver tumors by butter yellow (p-dimethyl amino azobenzene) are too familiar to necessitate recounting (710, 650) Among others a fruitful field would seem to be the relation of the inorganic elements to the development of epidermal tumors It has been shown that when methylcholanthrene is applied to the skin of mice there is a decrease

in the calcium, iron, zinc, and copper contents of the epidermis (14). It would be of interest to determine the effect of deficiencies of these elements on the development of cancer in the epidermis of this species.

The *geneticist* also should find the field of nutritional deficiencies of interest. For instance, there is some evidence that hereditary hypotricotic rats will respond to cysteine feeding by growing hair (278). The behavior of certain congenital waltzing and dancing mice (15) is reminiscent of descriptions of the activities of valine-deficient rats. Then, too, the breeding of vitamin susceptible and vitamin resistant strains would seem to offer great possibilities for the geneticist and nutritionist to collaborate. There is already evidence that this can be accomplished. In the development of both rickets (378) and dental caries (16) significant differences have been observed in various strains of animals. Such evidence may help explain variations in response to deficiency of certain nutrients, such as the response of the kidney to choline deprivation. The field of genetics is one whose relationship to deficiency studies has been, up to now, little explored.

Lastly, nutritional deficiencies should be of particular interest to the *comparative pathologist* and *veterinarian*. Although the response of the rat to depletion in single essential nutrients has been extensively studied, such is not the case for the guinea pig, mouse and certain domestic animals. The reactions of several species to deficiencies of all of the essential nutrients should be systematically examined. Another fertile field would seem to be that of spontaneous or endemic deficiency disease, which is in the domain of the veterinary pathologist. Deficiencies of calcium, phosphorus, iron, copper, and cobalt all fall into this group. Studies already carried out have yielded valuable information. However, such investigations are based on fairly impure rations of unknown dietary content and should be repeated with diets of known composition. A notable example, of course, is copper deficiency.

These few examples should indicate some of the ramifications which research in nutritional deficiencies has in other fields. Obviously, a most important part of the study of tissue changes associated with a lack of dietary essentials is in the domain of biochemistry, and the correlation of physiological and morphological alterations must always be stressed. It is fitting to close this introduction with a plea which Wolbach has made to the biochemist and nutritionist. "A general pathologist who studies life chiefly from the morphologic aspects may well be appalled by the wasted opportunities represented by animals consigned to incinerators at the completion of carefully conducted experiments in nutritional fields" (379).

Specific and Non-Specific Tissue Changes

It is extremely important to realize that virtually any dietary restriction leads to some change in one or more tissues. The question which then arises

is whether the observed change is a specific or non-specific one. By merely reducing the caloric intake of the growing organism, growth may be retarded or completely stopped; in time certain characteristic alterations may be found at autopsy. If all of the dietary essentials have been present and have been utilized, one can ordinarily assume that the changes observed are non-specific, that is they are the result of inanition or athrepsia.

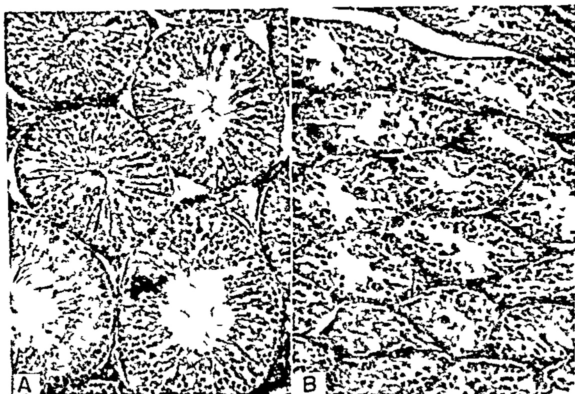


FIGURE 1 Testis. An Effect of Inanition. A. Normal testis showing spermatogenesis with spermatozoa in the lumen of several tubules which are normal in size. B. Testis from rat whose growth was impaired by inadequate caloric intake. Note difference in size as compared with A and also decrease in spermatozoa and spermatocytes. There are a few giant cells in the lumens of these tubules. Only Sertoli cells and a few spermatogonia remain.

At autopsy, in the athreptic organism, there is usually a marked decrease in fatty tissue, not only in the subcutaneous region but about the mesentery, kidneys, uterus and testes. The lymphoid tissue becomes atrophic so that the lymph nodes and spleen are greatly reduced in size. In addition there is marked diminution in the size of the thymus which is one of the best indices of nutrition in the growing organism. There may be alopecia. Furthermore there is usually atrophy of the genital organs with absence of spermatogenesis and ovogenesis. The vaginal lining becomes atrophic and reproduction is impaired. There may be complete cessation of osteogenesis so that the epiphyseal cartilage stops proliferating and bone growth ceases at the cartilage shaft junction and in the shaft as well. In the adrenal there may be loss of

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tion is exemplified in swine suffering from either acute or chronic thiamine deficiency the former animals may die, ostensibly of heart failure, with virtually no microscopic changes in the myocardium, while the latter usually exhibit extensive areas of damage in the cardiac musculature (506) Similar differences have been noted in many other deficient states

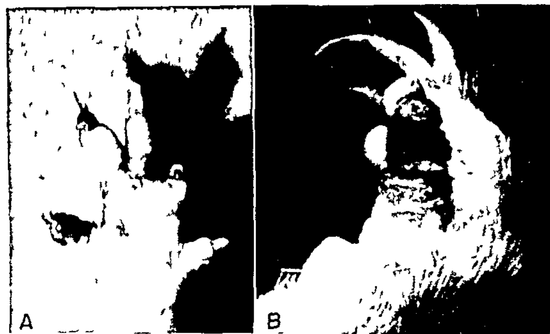


FIGURE 7 An Effect of a Multiple Vitamin Deficiency (299) The paws of animals which were placed on a synthetic diet to which thiamine and riboflavin but no other portions of the B complex were added *A* There is gangrene of the terminal phalanges of the first and second digits and an almost complete amputation of the fourth digit *B* There is localized gangrene of the first digit the others show no evidence of involvement (Courtesy of Dr Maurice Sullivan and *The Journal of Investigative Dermatology*)

An approach which has been used but little is to combine deficiencies of several essential nutrients and study the resulting syndrome This has been done with two or more vitamins with elements and vitamins or with several elements, interesting results have been obtained For instance, when rats are made *deficient in all of the B group except thiamine* skin lesions characteristic of pantothenic acid (606), pyridoxine (642) and riboflavin (552) deficiencies do not develop, on the contrary there is only atrophy of the epidermis and its appendages (298) Again when only thiamine and riboflavin are fed as the B group to rats gangrene and spontaneous amputation of the digits may appear (299) In another study animals were made deficient in an element potassium and a vitamin, thiamine, separately these two nutrients lead to myocardial necrosis but the hearts of rats deficient in both together showed no changes (96) Lastly, when a deficiency of sodium and chorine is produced simultaneously the results are different (107) from those

stainable lipoid in the cortex, especially in the glomerular and fascicular zones These, then, are some of the more striking changes which may be observed Other less dramatic but non-specific alterations have been described and are fully discussed by Jackson (17)

Again and again in the literature changes such as those described above are cited as evidence of specific damage which has resulted from deficiencies of various single nutrients Certain investigators seem to have overlooked the possibility that failure of an animal to eat or utilize his food may lead to many of these non-specific changes

On the other hand there are specific tissue alterations which are dependent on the absence of one or more essential nutrients from the diet Some of these lesions may only be produced by a lack of a single nutrient, others are common to deficiencies of more than one material The effect of vitamin C deprivation on the bones furnishes a good example of the first type, while corneal vascularization in the rat exemplifies the latter, where deprivation of a number of factors each one an essential nutrient causes capillaries to invade the avascular cornea In virtually all of the deficiencies that have been adequately studied, specific pathological changes both physiological and morphological have been observed Undoubtedly others will come to light as more intensive observations are carried out, especially when the newer techniques of histochemistry are utilized

The criticism raised above that the pathologist does not properly control his experiments can also be leveled at the biochemist There are numerous experiments some of which will be cited in the pages to follow in which the effects of inanition and differences in weight gain were not considered in evaluating the results

It is extremely important to mention certain other pertinent points in regard to the actual techniques of nutritional experiments One way of reducing the error due to inanition is not to feed control animals *ad libitum*, but to use the paired feeding technique In this procedure the control animals are given an amount of diet equal in weight to that which the deficient animal ate the day before An even stricter method though more laborious and one which has been little used is to attempt to manipulate the dietary intake of the controls so as to produce the same gain or loss of weight manifested by the deficient animals Another extremely important point which has been emphasized by Wolbach (317) is the study of reparative phenomena in deficient animals to which a missing essential nutrient has been administered for varying periods before autopsy

Furthermore it is important to realize that when an acute overwhelming deficiency state is produced the morphological changes may be slight or even absent in comparison with tissue alterations which may be encountered when the deficiency is more chronic and therefore not so severe This situa-

vitamin A in the liver, and as a consequence the vitamin A concentration in the blood is diminished So too, when the thyroid gland is poisoned by thiouracil it is unable to utilize inorganic iodine to form physiologically active organic forms

D *Increased Excretion* Ingested materials may be absorbed normally, but reexcreted too rapidly to effect their necessary function Such conditions may occur when polyuria due to a variety of causes is present Sweating is another example and endocrine imbalance, such as hypoadrenalism with loss of sodium or the reverse with loss of potassium, may lead to disastrous results Parathyroid imbalance also promotes excessive loss of calcium and phosphorus from the organism Lastly, lactation is too often overlooked as a factor leading to a loss of one or more dietary essentials

E *Increased Requirements* Certain intakes of essential nutrients are adequate for the normal needs of the body, but occasionally the needs are increased due to a variety of causes and unless these requirements are met the deficient state may develop Fever, which results in an increased metabolism is prominent Hyperthyroidism is of course, another fairly common example Pregnancy and excessive growth both require an excess of certain nutrients over the normal intake

F *Inhibition by "anti" Substances* Certain materials which are closely related in structure to both vitamins and amino acids will block the action of these specific essential nutrients For example analogs of ascorbic acid nicotinic acid riboflavin and phenyl-alanine when fed in the diet will lead to evidences of deficiency in these specific materials (18)

All of the factors just cited (A to F) presuppose interference with utilization of nutrients whose sources are exogenous There are of course other endogenous essentials The question of whether such substances should be called hormones need not detain us here For instance whether the ascorbic acid which is formed by the rat is a hormone for that species, while it is a vitamin for man and the guinea pig are questions beside the point It should be borne in mind however, that the list of essential nutrients is doubtless by no means complete Enough stress cannot be placed on the role which the micro-organisms that compose the intestinal flora play in the elaboration of certain nutrients Their importance in the manufacture of vitamins is too well known to warrant further discussion They may also be a source of certain amino acids a question requiring careful consideration (page 73) One can confidently predict that the entire question of essential nutrients for a given species will not be settled until representatives of that species are raised in sterile environments so as to eliminate the role of micro-organisms completely Although a start has been made with sulfa drugs the administration of such compounds introduces another unwelcome variable

which occur when either one or the other of these essential nutrients is withheld from the diet (110, 122)

Since the advent of purified rations and crystalline vitamins few studies of inanition have been reported, here would seem to be a fruitful field for future investigations

Endogenous Nutritional Deficiencies

The single essential nutrients which were alluded to at the beginning of this section must all be furnished from exogenous sources—at least for some organisms—if physiological and/or morphological evidences of damage are to be averted Naturally, the experimental approach eliminates one or more of these nutrients from the diet of the animal, one may then study the resultant physiological and morphological changes which take place Such a situation of course, does not ordinarily occur in man unless produced deliberately or because of economic reasons There are, however, a number of subsidiary and contributory factors which can lead to experimental dietary deficiency in animals and to natural disease in man These situations deserve mention

A *Interference with Intake* Loss of appetite (anorexia) due to a variety of causes may lead to a deficient intake of one or more nutrients Gastro-intestinal disease or other metabolic disturbances including pregnancy or food allergy may produce anorexia Then too, certain mechanical factors may be important Tumors within or without the intestinal tract may lead to partial or complete obstruction Lastly, adentia inflammation of the buccal tissues et cetera, may interfere with the ingestion of foodstuffs

B *Interference with Absorption* Although adequate amounts of an essential nutrient may be ingested optimal quantities may not be absorbed due to a variety of reasons Hypermotility of the intestinal tract may move the material through the lumen too rapidly for adequate absorption to take place, or insoluble complexes may form and prevent absorption of a particular material such as the combination of lead or beryllium with phosphorus and oxalate or fat with calcium Absence of digestive secretions may likewise inhibit absorption, the efficiency of bile and pancreatic juice for the absorption of the four fat-soluble vitamins is an excellent example Lastly, certain essential nutrients may actually be destroyed or inactivated before absorption from the intestinal tract The destruction of thiamine by an enzyme from certain fish and the inactivation of biotin by avidin are examples of these complications

C *Interference with Storage or Utilization* Even after adequate amounts of one or more nutrients are ingested and absorbed, they may be poorly stored or utilized Hepatic disease for instance may lead to low levels of

tion of the characteristics of the red blood cells and examinations of the cornea by the low power of the slit lamp Finally, however, tissues must be removed for microscopic examination, whether during life or after death, before gross lesions make their appearance In time the latter occur and diagnosis may be made from the macroscopic or clinical findings

The above sequence, of course, does not take place in every instance, nor does it go on to completion so that one will find gross or even microscopic lesions in every deficiency—quite the contrary In thiamine deficiency of swine for instance, an animal may die of heart failure, having previously shown electrocardiographic abnormalities, at autopsy virtually no microscopic changes may be found in the heart (506) The above concept of the pathogenesis of dietary deficiencies serves a useful purpose, especially in experimental studies of nutritional disease For a fuller discussion, especially from the clinical standpoint, one should consult the paper of Dann and Darby (2) It must be emphasized that no single nutrient has been simultaneously studied from the biochemical, physiological and morphological standpoints—something which is greatly to be desired

The Pathogenesis of Nutritional Disease

Bearing in mind the various factors which may affect the absorption, utilization, excretion, et cetera, of the essential nutrients what sort of a picture may we draw of the usual course of events which may be expected to occur as the deficient state develops? Although there are certain obvious exceptions which will be alluded to later most workers (2) in the field of nutrition have adopted the hypothesis that the physiological and pathological changes which result from deficiencies of essential nutrients develop in a definite and orderly sequence *1* Decreased concentration in the blood and intercellular fluid *2* Decreased intracellular concentrations in one or more tissues *3* Physiological changes in such tissues followed by *4* Pathological alterations which are first seen microscopically and then become grossly visible

It has been assumed that a decreased blood concentration of an essential nutrient is evidence of a decreased saturation of the body in that nutrient Such a concept is of course based on normal' or lower limit of normal values which unfortunately have been extremely difficult to determine Chemical studies of blood plasma are therefore not entirely satisfactory, much more useful than blood plasma are saturation or desaturation tests which lead one to the second link in the pathogenesis of deficiency disease decreased concentrations of the nutrient in one or more tissues

Here one is on firmer ground since the actual concentration of a given nutrient can be measured in red cells the white cell-platelet layer, muscle biopsies and, of course almost any tissue from an experimental animal at autopsy So too, histochemical studies can be made of tissue sections and decreases in nutrients such as vitamin A (310) or riboflavin (760) may be demonstrated under the microscope When the concentration of a particular nutrient in a certain tissue falls to a critical level, one may then expect evidences of metabolic derangements to appear These may manifest themselves in a variety of ways which are amenable to detection and measurement Abnormal metabolites may be found in tissues blood or excreta pyruvate (507) xanthurenic acid (635) and parahydroxyphenyllactic acid (454, 455) are examples of this phenomenon Liver function tests may be employed to detect changes in that organ (677) Then too physiologic measurements of normal processes can be employed electrocardiogram (504), electroencephalogram (531) tests of dark adaptation (321 339) and blood pressure determinations (111)

When the concentrations of a given nutrient have reached certain minimum tissue levels which are incompatible with life morphologic alterations may be expected This, however does not necessarily mean that the entire organism dies Tissues may be examined before death counts and determina-

tion of the characteristics of the red blood cells and examinations of the cornea by the low power of the slit lamp Finally, however, tissues must be removed for microscopic examination, whether during life or after death, before gross lesions make their appearance In time the latter occur and diagnosis may be made from the macroscopic or clinical findings

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PART II

THE ESSENTIAL ELEMENTS

"That the sodium, potassium, calcium, magnesium, phosphate, chlorine, and sulphate ions play an essential role in living protoplasm is a fact generally accepted. That the ion-protein compounds determine the peculiar properties of the membranes of living tissues is highly probable, and the constancy of composition of these combinations of ions with proteins in most of the body tissues is maintained only when the concentrations of the several ions in the liquid medium, the blood and lymph, remain constant within certain limits. The sensitiveness of the heart muscle to 'unbalanced' salt solutions, and of eggs developing in sea water to which a single basic ion as magnesium or potassium has been added in excess suggests that even slightly abnormal relationship between certain ions in the blood, if maintained for a prolonged period, may prove detrimental to the higher organisms." McCollum and Davis 1915 (762)

Part II

THE ESSENTIAL ELEMENTS

Introduction

About one percent of the organism's mass is composed of inorganic matter, that is, the metallic and non-metallic elements. Some occur in much larger quantities than others, the latter are usually called trace elements since they are found in such minute amounts. For our purposes the 96 elements which are now known may be divided into three groups: those which occur in the tissues and have been proved to be essential for one or more species, those which are found in varying amounts in the animal organism, but which are thought to be dispensable, and those which have not been demonstrated in the organism under normal conditions. The last group can be eliminated from any further discussion.

Eighteen elements comprise the first group: carbon, hydrogen, oxygen, nitrogen, sulfur, calcium, magnesium, sodium, potassium, chlorine, phosphorus, iron, copper, cobalt, zinc, manganese, iodine and fluorine. The position of the last element is not as certain as that of the others. In the second category are those elements which have been suspected to be necessary for the integrity of certain mammalian tissues since some are usually found on repeated analyses of tissues or secretions such as milk and since a few, for instance, boron and silicon, are necessary for plant growth. The presence of many of this group of elements in foodstuffs doubtlessly explains their occurrence in the organism. Such include arsenic, lead, rubidium, cesium, strontium, tin and barium, a more detailed discussion of which will be deferred until later.

Function of the Essential Elements

The elements play several important roles in the organism and undoubtedly have other functions as yet unknown.

In the first place they serve as structural components of tissues and as sources of energy for cellular metabolism. The place of hydrogen, carbon, oxygen and nitrogen in these connections is obvious since they are the constituents of water, carbohydrate, protein, and fat. Among the other essential elements, calcium and phosphorus in particular have a prominent place in giving structural stability to the bones and teeth. Smaller amounts of the other elements are found in these two tissues as well.

The indispensable elements serve in the regulation of acid-base equilibrium, both intra-cellular as well as extra-cellular. The relations here are too familiar to require further notice (49).

Perhaps even as important is the specific role of many of the essential elements as an integral part of certain enzymes or as activators of enzymatic reactions. Some examples might make this more clear. Zinc, for instance, is known to be a constituent of two enzymes, carbonic anhydrase (175) and

uricase (176) copper is said to be a constituent of ascorbic acid oxidase (128) Then too a large group of enzymatic reactions is specifically activated by certain elements For example, calcium is necessary for the reaction prothrombin→thrombin (440), while potassium activates the phosphorylation of creatine (83) Manganese is an important ion for the activity of arginase (166), while magnesium is an integral part of the cocarboxylase system (53) These are but a few examples of the importance of inorganic elements in enzymes and enzymatic reactions In addition, of course, the presence of iron and copper in certain oxygen carriers should be recalled (128)

Isotopes

Mention might be made of the occurrence of elements having the same atomic number but different atomic weights, that is isotopes Little investigation has been directed at a study of the possible natural occurrence of the isotopes of various essential elements in the animal organism These investigations are of some theoretical importance as is exemplified by one such study on the comparison of the distribution of K_{19}^{39} and K_{19}^{41} in potassium chloride and the tissues of rats (19) Most of the tissues had the same ratio of the two isotopes as compared with the inorganic compound with the exception of bone marrow and blood plasma Here there was a slight but significant increase in the heavier isotope Whether one isotope of a given element is physiologically more reactive than another must be left for further investigation

A more important phase of the subject of isotopes is the use of radioactive elements in metabolic studies Such forms of virtually all of the essential elements have been prepared and are being utilized Some examples of their use will be cited in the pages which follow

Ubiquitous Elements of Unknown Function

As was noted above there is a group of elements whose presence is indicated in the organism by chemical methods of examination but whose indispensability has not yet been proven Although a few of these elements may be shown to be essential at some later date it is likely that most to be discussed are ingested with the food or inhaled into the lungs and are therefore purely *fortuitous* The *more important of these will be mentioned* below, the data are based for the most part on spectrographic analyses of tissues and milk

Since *boron* is essential for the growth of plants (764) this element has been investigated with respect to its indispensability for animals Several independent investigations have failed to reveal any evidence that boron is an essential element for the rat and the conclusion must be drawn that if

this element is necessary for this species the amount needed is less than 6 micrograms per rat per day (20, 21, 763) Boron has been identified in milk (22 23) but not in the tissues of the newborn rat (25) Based on growth studies in rats it is said that boron will replace potassium in a diet deficient in the latter element (95), we have been unable to confirm this observation (652)

Aluminum has been investigated in some detail Traces are found in milk (22) and in the tissues of the newborn rat (25) However, studies indicate that if this element is needed extremely small quantities must be available since one microgram in the milk diet employed in one investigation is sufficient to promote normal growth (26)

Silicon of course is necessary for the growth of certain plants (27), and is a fairly common constituent of most tissues (24, 25) and milk (22) No studies have been reported dealing with its indispensability for Mammalia

Bromine is present in rather large quantities in the blood urine and tissues (28) There is no evidence available that this element is or is not an indispensable one a problem which should be studied

Arsenic, too is found in tissues and milk Two micrograms a day of this element are sufficient to supply the needs of growing rats (192), rations lower in arsenic content than this have not yet been devised

Rubidium is a common constituent of blood and tissues (24) No studies have been reported in which a deficiency of this element has been produced The present writer has shown that rubidium will partially substitute for potassium in a diet deficient in the latter element, since certain lesions are characteristic of potassium deficiency fail to appear when rubidium is added to the diet (94) The substitution is not a complete one, of course

Cesium has been demonstrated in the retina of the ox (29), but not in milk The function of cesium in the eye has not been elucidated When this element is substituted for potassium in a potassium-deficient diet it partially protects the heart and kidneys from the effects of potassium deprivation (94) Another element *barium*, has been demonstrated in the eye of oxen, where it is present in the choroid in a concentration of 1 5 per cent of total dried tissue (30)

Vanadium which is found in milk (22) but not in the tissues of the newborn rat (25) has been investigated with respect to its indispensability If this element is necessary it must be present in quantities less than 1-5 parts per million of diet so that much more purified diets will have to be concocted before the question of its indispensability can be settled (31)

Certain other elements are also found in tissues or milk but have not been carefully studied These include *lead* (32), *lithium* (22) *strontium* (22) *tin* (32) and *titanium* (23)

The above group of elements which is an incomplete listing should give

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is available on others. It has been shown, for instance, that sodium will partially replace potassium in the tissues of animals depleted in the latter element (78). It is possible that sodium attempts to correct the acid base balance of the cells; certain other functions of potassium, such as the maintenance of the integrity of heart muscle and kidney, do not appear to be affected, however. Two other elements of this group, rubidium and cesium, more nearly replace potassium, at least for a short time (94). When the former is added to a potassium-deficient diet, the characteristic necrosis of myocardial fibers and changes in the renal tubular epithelium fail to appear. The animals die, however. Cesium, which has a higher atomic weight than rubidium, only partially protects against cardiac and renal damage. Since boron and potassium seem to be inter-related in plant metabolism, observations have been made on rats placed on potassium deficient diets to which boron has been added (95). From the data reported, weight gain is better in the animals whose potassium depleted diets contain boron than those whose rations do not contain the latter element. The present writer has failed to confirm these observations, however, and further finds that characteristic lesions appear in the myocardium and kidney of animals on a potassium deficient diet supplemented with boron (652).

Bone, of course, furnishes another excellent example of the interchangeability of its constituents. Strontium, magnesium, bismuth and lead may replace calcium in the inorganic structure of bone. Substitution of the latter element is of great clinical significance in the X-ray diagnosis of lead poisoning, particularly in children (191). Here there is an increase in the number and size of the spicules of calcified and plumbified cartilagenous matrix material. Inasmuch as there is a fairly large quantity of lead present, and since the absorption coefficient for x-rays varies approximately as the fourth power of the atomic number of an element, it is obvious that such a region will produce a bright zone at the cartilage shaft junction of the bone in the roentgenogram.

The Essential Elements and the Periodic Table

It is probably premature to attempt any explanation of the relationship of the nutritionally essential inorganic elements to their places in the periodic table. The subject is an interesting one, however, and has aroused no amount of philosophic speculation. A periodic table of all the elements is presented in Table II. The indispensable elements are printed in bold face, those which are indispensable for plants in italics, those which are ubiquitous in normal type, while those which have not been found in the organism are in small type. It is only obvious that many which are in the indispensable group are found on either side, that is, in the most reactive portions of the periodic table. But this does not aid in explaining the place of lithium, which

some idea of our knowledge at this time. Undoubtedly, as more precise methods are developed, some of the elements just enumerated may be shown to be indispensable. The use of the hydroponic technique to grow foodstuffs in media uncontaminated by certain elements would seem to be worthy of application to this problem (214).

Inter-relations of the Elements

An interesting aspect of the study of the dispensable and indispensable elements in nutrition is that phase which deals with the substitution of one for another in physiological processes. A good deal is known of those elements comprising the alkali earth group in this regard but little information



Figure 5. An Example of the Interrelation of the Elements. This is a section from the cartilage shaft junction of a rib from a colored girl two years of age who ate the paint from her dolls for two months. She had the usual clinical story of convulsions, lead line in the bones by x-ray, elevated blood lead (5 mgm. per cent), increased C.S.F. pressure and positive Pandy. At autopsy characteristic intranuclear inclusion bodies were found in the liver and kidney together with widespread changes in the central nervous system. The bone shows a tremendous increase in the zone of cartilaginous matrix material. Compare with the normal Figure 27, page 10. This zone is composed of lead which is partially replacing calcium in this region. The persistence of such a dense zone may be due both to a continued ingestion of lead and an inability of the organism to destroy such tissue as readily as possible. H. and E. ×15. (191)

hemorrhage, prolongation of the coagulation time, inflammation of the gastrointestinal tract and osteoporosis '

Biochemical Relationships Besides its major role as a component of the skeletal system where more than ninety percent of the organism's total calcium is found, the ions of this element are necessary for certain well-known physiological processes Shortly after Ringer (37) announced the importance of calcium in the contraction of heart muscle, its role in the coagulation of the blood was demonstrated (38) The mechanism of calcium ions in this phenomenon is still somewhat obscure, although an hypothesis that the element is necessary to unite two factors, A and B, to form prothrombin promises to clear up some of the divergences of opinion (440) The effect of calcium ions on the irritability of nerve and muscle and its relation to tetany should be recalled There is some experimental evidence that calcium controls the permeability of capillaries by virtue of its enhancement of the solubility of intercellular cement substance," which may be a calcium proteinate (40 41)

Pathological Effects Despite the importance of calcium for the organism knowledge of the tissue changes associated with deficiency of this element are woefully inadequate Low calcium rickets has of course been produced although no descriptions are available in animals whose diets also *contain optimal amounts of vitamin D The general pathology of rickets is* discussed elsewhere (page 104) Aside from chemical alterations which will be summarized below calcium deficiency in both rats and dogs leads to hemorrhage lesions of the gastrointestinal tract and of course, rickets

The most severe calcium deficiency has been reported in rats by Boelter and Greenberg (42 43) When such animals are placed on a diet containing only 0 01 percent calcium growth is retarded in from four to five weeks and after seven to ten weeks the animals exhibit a generalized decreased sensitivity and reactivity Coincident with this the serum calcium falls to about five milligrams per hundred centimeters, tetany however, does not appear Paralysis of the hind legs may be noted and, when the deficient animals are stimulated by galvanic shocks collapse occurs Sixty percent of the rats succumb by the twenty-third week At autopsy widespread hemorrhages are found in the tissues, extravasation of blood is prominent in the nervous system especially in those animals which exhibited paralysis before death Hemorrhage and paralysis are common in the young born of calcium-deficient females (39) and bleeding is also a prominent feature of the calcium-deficient syndrome reported in dogs (36) It is unfortunate that microscopic studies have not yet been reported in these two species, since it would be of interest to determine if possible whether there is actual damage to capillary endothelium or whether the hemorrhages are incident to normal trauma to vessels That the former may be the case is suggested by the work

Table II

ABRIDGED PERIODIC TABLE OF THE ELEMENTS

GROUP	I	II	III	IV	V	VI	VII	VIII
PERIOD								
1	H							
	Li	Be	*B*	C	N	O	F	
2	Na	Mg	Al	*Si*	P	S	Cl	
3	K	Ca	*Sc*	Ti	V	Cr	Mn	Fe Co Ni
	Cu	Zn	*Ga*	Ge	As	Se	Br	
4	Rb	Sr	Y	Zr	Cb	*Mo*		
	Ag	Cd	In	Sn	Sb	Te	I	
5	Cs	Ba						

is toxic and beryllium which appears to be inactive. The reason for the appearance of the essential elements in the periodic table has been explained on the basis of subshell of transition, atomic number and rank of the elements (787). Whether such an explanation is a valid one remains to be seen.

Calcium*

Historical. Innumerable experiments utilizing calcium-deficient diets have been reported; for the most part, however, the rations which were employed have been deficient not only in calcium but in other essential nutrients as well. In 1937 Martin (36) prepared a diet adequate in other respects but containing only thirty parts of calcium per million. The characteristic syndrome which develops in dogs fed this ration consists of widespread

* Since life is impossible for Mammalia without carbon, hydrogen, oxygen and nitrogen these elements will not be considered in this book.

has been corroborated by the appearance of tetany and reduction in serum calcium concentrations.

In summary, experimental calcium deficiency leads to a derangement of blood coagulation and of the integrity of capillary epithelium. Tissue changes consisting of ulceration of the stomach, cataracts and parathyroid enlargement have been described.

Calcium Deficiency in Man. The most dramatic pathologic manifestation of calcium deficiency in the human—rickets and osteomalacia—are described in the section dealing with vitamin D (page 104). Another form of calcium deficiency which is followed by disastrous results in both man and animals is, of course, produced by removal of the parathyroid glands.

Magnesium

Historical. The spectacular syndrome of magnesium deficiency in the rat was first reported by McCollum and his co-workers in 1931 (47). Subsequent studies by the Johns Hopkins investigators and others have aided in clarifying some of the changes which take place in the animal organism when dietary magnesium is restricted.

Biochemical Relationships. Magnesium is widely distributed in the tissues where its intracellular concentration is only secondary to potassium. The greatest concentrations are found in the bones, although this cation accounts for only 0.5 to 0.7 percent of the ash (48). Magnesium is also present in plasma, where it furnishes only a very small proportion of the basic ions (49). The irritability of muscle and nerve is affected by changes in concentrations of magnesium; excesses produce narcosis (50), while decreases lead to hyperirritability (51). In addition, magnesium ions, like those in certain other elements such as potassium and zinc, are necessary for the activity of certain enzymes, for instance phosphatase (52) and cocarboxylase (53) (489), although this latter action is not entirely specific since manganese will substitute for it. Magnesium appears to participate in virtually every phosphorylating mechanism.

Pathological Effects. In rats (54) and dogs (55) placed on a diet containing only 0.18 percent magnesium, McCollum et al. have described the development of a specific syndrome characterized by dilatation of the cutaneous vessels, hyperirritability and convulsive seizures. The latter may be precipitated by external stimuli of various types. The first attack proves fatal in about eighty percent of rats or dogs. The following description graphically portrays the course of one of the seizures. "The excitable animal (rat) startled by sound, races at rapid speed in a wide circle until

of Chambers who has presented evidence that "intercellular cement substance" may be a calcium protein complex, if this is true one would suppose that there is actual damage to capillaries in calcium deficient animals (40 41) The explanation of hemorrhages in calcium deficiency is analogous to the situation in vitamin K deficiency where a similar question remains to be settled (page 128)

Boelter and Greenberg (42) have not described any abnormalities in the gastrointestinal tract of the rat, however, other observations in this species are not in agreement since lesions are described in the antrum of the stomach though not in the fundus or rumen (44), such changes consist of hyperplasia of the lining epithelium with necrosis and hemorrhage It is of interest that in dogs an 'inflammed hemorrhagic gastric and intestinal mucosa with occasional ulceration" has been described (36)

The reproduction of rats on a calcium low diet has also been studied (39) Fertility rapidly decreases and the animals soon fail to mate In addition in those females which give birth to young there is insufficient milk to nourish their offspring Whether these abnormalities are the effects of inanition are questions which remain to be investigated

A most interesting observation is that moderate amounts of calcium salts injected into normal rats are perfectly innocuous while intravenous administration of similar quantities into calcium deficient animals results in rupture of the right ventricle of the heart (42)

The neurological disturbances of calcium deficient animals are not at all clear Tetany does not appear to occur but paralysis, particularly of the hind legs has been noted in both rats (42) and dogs (36) In addition tonic clonic convulsions are said to occur in the latter but not the former species Studies both physiological and anatomical of the nervous tissues of calcium deficient animals are greatly to be desired

Several other miscellaneous effects of calcium deficiency have been described and should be studied further In the rat unpurified, low-calcium diets lead to an increase in size of the parathyroid glands, the change is said to be due to both hyperplasia and hypertrophy of the cells, an increase in the number of osmophilic cells and in the complexity of the golgi apparatus have also been noted (45) Such alterations are, of course, in keeping with the current concepts of parathyroid activity in relation to levels of blood calcium and explain the increased activity of these glands in cases of renal insufficiency in which the levels of serum calcium may be reduced

When rabbits are placed on a low calcium diet lens opacities have been noted (46) Such changes in the lens may be observed during the second week of the deficiency and consist ophthalmoscopically as slits, vacuoles and dots near the equator of the lens The opacities then progress out toward the anterior and posterior suture lines Calcium deficiency in such animals

leaps into the air at the same time spinning laterally several times, or it may "curl up" with marked flexion of all extremities, or it may do neither. There is marked cyanosis. Associated with the convulsive seizure is regurgitation of the stomach contents into the esophagus and mouth, as sacrifice experiments during this period have shown.

Within a short time the animal rears from the dorsal or lateral recumbent position in an attempt to stand but its extremities will not support it. The animal buries its head in its outstretched fore limbs and propels itself forward entirely by its hind limbs which, however, are so extended with paws hyperextended that the dorsal, not the plantar surface, bears the weight. Instead of forward motion fine tremors may appear over the body. Throughout this stage the eyeballs are retracted.

Following the convulsive stage comes the recovery stage, doubtless dependent on exhaustion. During this period there is moderate cyanosis of skin, coldness of the extremities, lacrimations from the dull, shrunken eyes, champing of the jaws and drooling from the mouth. A hemorrhage may issue from the nose and orbit and bloody, frothy fluid consisting largely of regurgitated stomach contents mixed with blood may bubble from the mouth. No urinary or fecal incontinence is seen during the attack."

Microscopic examination of the nervous tissues of magnesium-deficient animals have not been reported. Studies of the action of certain drugs on magnesium-deficient rats have lead Tufts and Greenberg (56) to conclude that the site of action of the sensory stimuli which produce the seizures is in the midbrain. These investigators feel that tetany resulting from calcium or magnesium deficiency differ since the muscle spasms in the animals depleted in the former cation are abolished by curare, while those deprived of magnesium are not. In view of our inadequate understanding of the true nature of tetany it would seem unwise to argue that magnesium deficiency can or cannot lead to this syndrome. It is of interest, however, that the electrical threshold is reduced in magnesium-deficient rats as it is in tetany resulting from other causes.

Chemical studies on magnesium-deficient animals have shown an early and an abrupt fall in serum magnesium from a normal of 2.96 milligrams percent to 0.81 milligrams percent (57). After this initial fall the serum magnesium slowly rises. As would be expected the urinary excretion of the ion is greatly diminished; there is a concomitant retention of calcium (58). Certain other abnormalities in blood chemistry have been interpreted to result from a general nutritive failure since such changes occur later in the deficiency, for instance, increased cholesterol and decreased fatty acid values (57) together with a reduction in serum phosphatase activity (59). Changes in the bones and teeth will be discussed below.

McCollum et al. (54) have called attention to the appearance of tachy-

it finally falls on its side The entire body of the animal is now rigid with head stretched back fore limbs extended at three upper joints and flexed at the metacarpophalangeal joint and hind limbs extended backward So fixed are the jaws that often the tongue is perforated by the clenched teeth The skin presents a waxy appearance All respiratory movements cease during the attack and return with the relaxation of the musculature Priapism may appear at this time and persist until death

This stage of spasticity is succeeded by a period of relaxation lasting only a very short time While still lying on its side the animal exhibits



FIGURE 4 Magnesium "Tetany" (6) From right to left are shown several stages of a convulsive seizure which characterizes magnesium deficiency In the first stage there is great spasticity with hyperextension. This is followed by relaxation which may be in turn followed by rigidity and opisthotonos. The animal then may either recover or die (Courtesy of Dr Maurice Sullivan and the *Archives of Dermatology and Syphilology*)

twitching in various regions, or paddles rapidly with all extremities Coincident with this behavior the animal s eyeballs become more prominent the ears stiffen and project backwards against the side of the head and the fur stands erect Then reappears a tonic spasm in which the rigid body assumes a typical position with the fore limbs pressed tightly against the thorax fore paws clenched and hind extremities extended This spastic condition may give way to clonic contractions in which the fore limbs are alternately drawn up to the chest and extended from the body Next the animal suddenly



FIGURE 5 Magnesium Deficiency (63) *A* This shows extensive edema of the digits and the plantae of the paws In addition there is ulceration of the surface of the latter *B* Skin from tail to show hyperkeratosis, acanthosis and vacuolization of some of the epithelial cells There is diffuse cellular infiltration in the corium (Courtesy of Dr Maurice Sullivan and the *Archives of Dermatology and Syphilology*)

cardia as acute magnesium deficiency develops, electrocardio-graphic studies in rats reveal sinoauricular block (56)

Microscopic examination of the tissues of magnesium depleted rats have revealed changes in the skin kidneys liver, and teeth Dilatation of the cutaneous vessels is one of the prominent features of magnesium deficiency which McCollum and Orent (47) first described, such hyperemia lasts about a week In those animals which survive the ensuing convulsions edema of the paws and ears as well as changes in the skin may be noted Careful studies of the pathogenesis of the cutaneous lesions have been reported by Sullivan and Evans (60) From the fourth to the eighth day of the deficiency erythema and edema become prominent in the ears, paws and trunk Microscopically the vessels of the cutis are dilated, fluid and cellular infiltration are observed in the corium At this stage there is no alteration in the epidermis nor any loss of hair Later, however, a loosely laminated hyperkeratosis appears and is followed by a patchily distributed acanthosis Individual cells become vacuolated and display pycnotic nuclei No changes can be detected in the sebaceous and coil glands Sullivan and Evans (60) are unable to substantiate a claim (56) that signs of magnesium deficiency are affected by the vitamin B content of the diet nor can the contentions of MacCardle et al be confirmed On the basis of spectrographic (61) and micro-incineration (62) studies, the latter investigators find a decrease in the magnesium content of the skin in cases of human neurodermatitis From this they postulate that neurodermatitis and magnesium deficiency are identical or similar diseases Sullivan and Evans (63) have conclusively shown that the pathologic manifestations of these two syndromes are quite different

The renal lesions in magnesium-deficient animals have been inadequately studied The picture is further complicated by the use of diets which may not have furnished all necessary nutrients The following changes have been noted extreme degeneration of tubular and glomerular epithelium with calcareous deposits in the lumens of the tubules (64) calcium deposits in the straight and collecting tubules which lead to cystic dilatation of the structures above (65) "extreme degeneration of the tubules and glomeruli and deposits of calcareous material in areas of degeneration" (60) Increased urinary volume and proteinuria but no hematuria or casts have been observed Hypoproteinemia ensues and may be followed by edema (66) More complete studies of the renal changes are much to be desired Alterations which have been described in the liver are even more fragmentary and difficult to evaluate "hyperemia perivascular edema and occasional disintegration of liver cells" have been recorded (60)

Studies of magnesium deficiency by the Johns Hopkins investigators revealed changes at another site the teeth Kline et al (67) noted extreme hypertrophy of the gums the result of subepithelial connective tissue pro-

data concerning the potassium content of representative tissues of the rat the paper of Orent-Keiles and McCollum (78) should be consulted. This element may be demonstrated in muscle by histochemical methods. The procedure which employs sodium cobaltinitrite should prove useful in studies of experimental potassium deficiency (79).

Because of its presence within cells a great many physiological roles have been ascribed to potassium. Among the most important functions of this cation are its relationship to the metabolism of muscle and nerve. The participation of the potassium of extracellular fluid in the contraction of cardiac muscle was demonstrated in 1882 by Ringer (37) who showed that this cation reduces contractions and favors the relaxation of cardiac muscle fibers. Furthermore small variations in serum potassium are said to affect the response of the heart to vagal stimulation; here sensitivity is increased by a rise in potassium content of the extracellular fluid (81). Potassium is likewise concerned with the metabolism of striated muscle, for in familial periodic paralysis serum potassium falls during an attack; there is not, however, a concomitant increase in the excretion of this cation (82). *In vitro* experiments have shown that potassium is necessary for the phosphorylation of creatine (83). When nerve is stimulated there is a loss of potassium. The implications of this are not clear. Potassium accelerates the synthesis of acetylcholine by brain tissue in vitro (84). These, then, are a few representative examples of how potassium functions in the organism; many other manifestations of the action of potassium have been reviewed by Fenn (85).

Pathological Effects. Both physiological and morphological effects of potassium deficiency have been described in rats (86, 87, 88), mice (89), dogs (90), and calves (91, 92). In the former species 0.17 percent potassium in the diet appears to be the minimal amount which will support optimal growth (88). The tissues which are the sites of damage are the heart, voluntary muscle (dogs only), and kidneys.

Using diets containing only 0.01 percent potassium and adequate in all other respects, the present writer in association with Orent-Keiles and McCollum (87) has described disturbances in growth and lesions in the heart and kidneys of rats. Animals acutely deficient may die in the third or fourth week; rats which have been maintained on a ration somewhat less deficient in potassium live for as long as three hundred and twenty-seven days.

Heart. Grossly, after several weeks on the potassium-low regimen tiny gray opacities are observed in the ventricles of the heart. Microscopic studies of the myocardium reveal lesions in animals which have been on the deficient diet for as little as eight days. The myocardial fibers at this time lose their striation and become hyaline and necrotic; coincident with these changes the interstitial spaces are infiltrated by leukocytes. These lesions range in extent from tiny foci which early in the course of the deficiency involve

liferation Striations were noted in the dentine, as well as alterations in the ameloblastic layer The dental structures have been carefully studied by competent oral histologists (68, 69, 70, 71, 72) and the following changes seem well established An early manifestation is retardation of dentine formation particularly that of the labial surface, which is half or less the width of the lingual dentine Peculiar striations in the dentine appear which may be due to variations of growth similar to those which are seen in the bones The odontoblasts are responsible for the changes in the dentine since these cells become atrophic and are inclosed on all sides by dentine In a similar fashion ameloblasts atrophy, as a result enamel formation is retarded and the resultant covering is hypoplastic Calcified stones are also a prominent feature in the pulp of magnesium-depleted teeth Chemical studies have shown no great decrease in the absolute magnesium content of the rat's incisor (73) This is unlike the situation in bone where magnesium has been shown to decrease and calcium to increase in the early stages of the deficiency (74) In this respect magnesium may be unique in that it seems to be able to leave the bones without destruction of the latter taking place

Histological studies of the growing bones of magnesium deficient animals have not been reported Bone apparently serves as a reservoir for magnesium, when the blood level falls magnesium is made available and is replaced by calcium (75)

In summary, magnesium deficiency in experimental animals leads to disturbances of the neuromuscular and vascular systems and changes in the teeth liver and kidneys The latter two organs require more study

Magnesium Deficiency in Man The syndrome of magnesium deficiency in the human must be very rare However, a case of suspected deficiency of this cation has been reported in a child (76) Convulsions were observed at seven months of age but gradually decreased, at the age of 3 there was dizziness and by the age of 6 years a tremor had become worse so that he was unable to write Tetany was noted and the plasma magnesium was found to be low Magnesium therapy led to disappearance of dizziness and tremor which returned when treatment was discontinued More cases must be observed before this isolated report can be accepted as evidence of magnesium tetany in man

Potassium

Historical The indispensability of potassium was reported first in 1918 by Osborne and Mendel (77) who demonstrated a retardation of growth in rats which had been placed on a diet containing only 0 033 percent potassium

Biochemical Relationships All tissues contain intracellular potassium For

until virtually exhausted The alterations in the myocardium tended to be more extensive in those animals made to exercise than in controls not so treated (93) Inasmuch as sodium has been shown to enter the tissues to replace potassium when the organism is depleted of the latter element (78),



FIGURE 7 Heart Chronic Potassium Deficiency (87) Myocardium of animal which had been *on a potassium deficient diet for 377 days There has been widespread destruction of muscle* fibers with subsequent replacement by connective tissue Though a few inflammatory cells are present most of the nuclei which can be observed are those of fibroblasts H and E x150

investigations have been made in order to yield information on the replacement of this ion by other elements, such as rubidium cesium and boron When rubidium is substituted for potassium in a potassium-deficient diet, myocardial lesions do not appear although the animals die after a short while (94) Cesium protects the heart of some animals but not all (94) Low potassium diets supplemented with boron as boric acid or borax are said to permit longer survival of animals than those without added boron (95) the present writer has been unable to confirm this (652)

Since identical myocardial necrosis has been observed in thiamine-deficient animals (506) the effect of an acute deficiency in both potassium and thiamine has been studied (96) Lesions fail to appear even though the animals may live as long as thirty-one days The reason for this protective effect is not clear and requires further investigation



FIGURE 6 Heart Acute Potassium Deficiency (87) *A* Small focus in wall of left ventricle of rat on potassium deficient diet for nine days There is destruction of a few of the myocardial fibers with infiltration of cells most of which are mononuclears Numerous such small foci are found scattered *about in* the myocardium in the right and left ventricles at this time H and E x300 *B Section through* entire wall of right ventricle of animal on potassium deficient diet for 12 days This shows more diffuse infiltration and more extensive involvement of the myocardial fibers H and E x150

only one or two muscle fibers to large areas as much as two low-power microscopic fields in greatest diameter as the deficiency progresses In some hearts the tissues become diffusely infiltrated with leukocytes and are reminiscent of the lesions encountered in human myocarditis such as that following diptheria Alterations are found in both ventricles but are usually scanty in the auricular musculature Blood vessels are normal as are the epicardium and endocardium, no mural thrombi have been observed In animals living the longest there is usually an increased proliferation of connective tissue at the sites of necrosis of the myocardial fibers, so that scars of varying sizes are produced There is a reduction in the potassium content of the hearts of deficient rats (78)

The effect of exercise on the development of cardiac lesions has been studied by suspending weights to the thorax of rats and having them swim

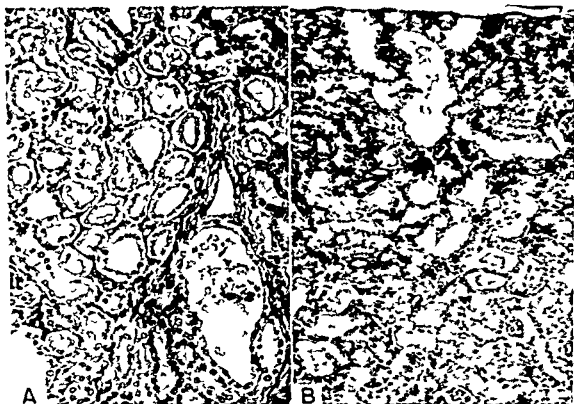


Figure 8 Kidney Acute Potassium Deficiency (87) A This rat had been on a potassium deficient diet for 15 days Note vacuolated cells in which fat may be stained and a dilated tubule which contains desquamated cells and debris Such tubular epithelium becomes necrotic and is sloughed off into the tubule H and E, x200 (Courtesy of the *American Journal of Pathology*) *B* Subacute potassium deficiency This rat had been on a potassium deficient diet for five weeks Note numerous dilated tubules lined by flattened regenerated epithelium Some contain hyaline staining material H and E, x150

cells become necrotic Cellular debris and fat globules are then found in the lumens of the tubules As early as the fourteenth day of the deficiency the tubules are found lined by flattened regenerating epithelium As time goes on there are many tubules lined by this type of epithelium and in some tubules the epithelium has become calcified Calcareous casts are found in the lumens of the tubules No glomerular lesions have been observed nor are there any changes in the renal blood vessels

Inclusion of rubidium in a potassium-deficient diet seems to protect the kidneys of all the rats so studied by the present writer cesium protects to a lesser extent (94) Excessive amounts of desoxycorticosterone as well as affecting the heart produce renal changes, lesions similar to those described as a result of potassium deficiency have been observed in rats and are made a little more severe as the potassium content of the diet is reduced (103)

In summary there is evidence from experimental animals that potassium deficiency leads to necrosis of the myocardial fibers and necrosis of renal

An observation intimately related to potassium and its importance to the integrity of the myocardium is that similar cardiac lesions may be produced by injection of desoxycorticosterone Such an observation is explained by the well-known action of this adrenal hormone to promote the excretion of potassium and facilitate the retention of sodium, lesions similar to those of potassium deficiency have been produced in rats by this means (97) When potassium-deficient animals are treated with cortical hormone, lesions appear sooner and are more extensive than when either one or the other procedures are employed (98) The heart muscle of animals treated with cortical hormone has a lower potassium content than normal (97) It is said that the hearts of animals treated with desoxycorticosterone develop lesions similar to those of rheumatic fever (99) Nothing resembling an Aschoff body has ever been encountered in our own potassium-deficient material nor do Selye's photomicrographs substantiate such a contention

Lesions resulting from potassium deficiency have been described in the myocardium of mice (89) as well as in the Purkinje network of the hearts of calves (92) Electrocardiographic changes substantiate morphological observation in the latter species (91)

In contrast to the cardiac lesions which have been produced in rats on low potassium diets, no alterations have been noted in the skeletal musculature (87) although the potassium content of such muscle is reduced (78) Even in rats which are forced to exercise strenuously, no structural alterations have been observed (93) Chemical studies however indicate that exercise *per se* does not significantly lower the potassium content of voluntary muscles (100) It is therefore, not unexpected to find that no lesions have been produced in striated muscle of the rat by injections of desoxycorticosterone (101) It is of some interest to note that when rats are simultaneously depleted of both thiamine and potassium necroses of the skeletal muscles appear (96) In contrast to this species paralysis has been described in dogs on purified diets of low potassium content (90) It is of further interest that periodic muscular weakness occurs when dogs are given excessive amounts of desoxycorticosterone

Kidney The only other tissue which is the site of injury in potassium-deficient animals is the kidney Changes have been described in both rats (87) and mice (89) In the former species microscopic lesions have been observed as early as the eighth day of the deficiency After several weeks the kidneys grossly appear pale and swollen As time goes on the organs increase in size and soon develop a finely pitted surface On microscopic examination the initial change to be observed is fatty infiltration of the tubular epithelium This begins with the appearance of small sudanophilic, non doubly refractile globules in the cytoplasm between the basement membrane and nucleus This deposition of fat continues and soon the epithelial

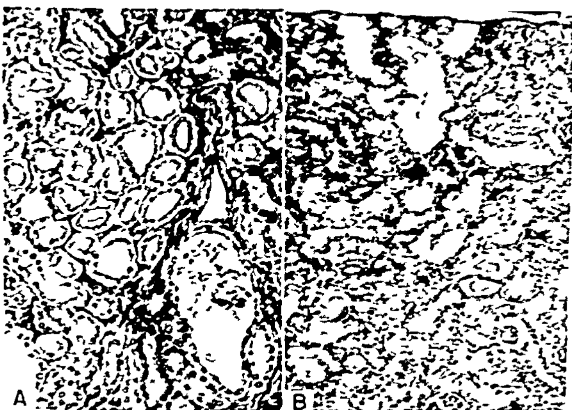


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stances of potassium deficiency in chronic nephritis (782) and diabetes mellitus (783) have been reported Such were characterized by muscular paralysis electrocardiographic changes and decreases in the concentration of serum potassium The etiology is not entirely clear through diuresis and glycogenesis appear to be important in the latter, while the possible inability of the kidneys to form ammonia to excret in combination with acid radicles was suggested in the former group

Sodium

Historical Retardation in growth of rats on synthetic diets of low sodium content was first reported by St John (106) in 1926 the subject was more extensively studied some years later by Orent-Keiles, Robinson and McCollum (107)

Biochemical Relationships The majority of sodium in the animal organ is extracellular This ion accounts for 142 of the 155 milliequivalents per liter of the basic ions of the extracellular fluid (49) Except for evidence that sodium can replace potassium when the tissues are depleted of the latter cation (78) and its importance on the contraction of the heart (37) very little else is known of the functions of this element in the animal organism

Pathological Effects Orent-Keiles and McCollum (109) have devised a ration which contains only 0 002 percent sodium When rats are placed on such a diet gain in weight during the first few weeks is normal, growth then becomes retarded and in general after eight to ten weeks the animals either fail to gain or begin to lose weight All are dead by the eighteenth to the twenty-first week Grossly characteristic changes make their appearance in the eyes between the eighth and tenth week the corneae become cloudy and the lids appear swollen and lose their hair

The tissues of such deficient rats have been studied by the present writer in association with Orent-Keiles and McCollum (110) Microscopically, aside from non-specific athreptic changes in the reproductive system thymus and bone the only lesions to be found are those of the ocular apparatus The pathogenesis of these changes has been described as follows there is progressive dilatation of the ducts of the tarsal or meibomian glands This is apparently due to obstruction of their openings since granular pink-staining material is found attached to the lid margins Dilatation of the ducts accounts for the swelling of the lids which is noted grossly When the dilatation becomes extreme there is atrophy of the glandular elements Associated with these changes is an alteration in the character of the epithelium of the inner lining of the lids Normal columnar and goblet cells are re-



Figure 9 Kidney Chronic Potassium Deficiency (87) Kidney of a rat which had been on a potassium deficient diet for 84 days Section shows dilated tubules along the cortex and prominent dilated structures at the cortico medullary junction H and E ×16

tubular epithelium No other alterations have been noted other than those which may be ascribed to inanition

Potassium Deficiency in Man It is very unlikely that potassium deficiency in the human ever occurs as a result of inadequate dietary intake Conditioned potassium deficiency may be encountered however It will be recalled that injections of desoxycorticosterone lead to excessive excretion of potassium Toxic effects have been noted in man as evidence by electrocardiographic changes (104) and in one instance fresh necroses have been found in the myocardium (105) It will be recalled that a decrease in serum potassium occurs during attacks of familial periodic paralysis (82), electrocardiographic changes have been described in patients suffering from this disease (757)

A conditioned potassium deficiency occurs in infants with severe dehydration and diarrhea Other elements are of course lost as well but it has been shown that the parenteral administration of potassium ions causes more marked improvement than if this element is not given (765) Isolated in-

The effects of sodium deprivation have been studied in dogs in which observations were made over a period of eight weeks Loss of weight dryness of the skin and loss of hair are said to occur, the eyes show no changes (112)

In summary, sodium deficiency appears to lead to ocular changes in rats, the principal alterations are vascularization of the cornea and obstruction of the tarsal glands

Sodium Deficiency in Man Sodium deficiency in the human probably never occurs in an uncomplicated form, usually there is a concomitant deficiency of chloride as well Severe depletion of the sodium and chloride stores with an accompanying loss of water occurs in Addison's Disease which is due to destruction of the adrenal cortex with consequent inadequate cortical hormone production The urinary excretion of sodium and chloride is extreme under such circumstances Inasmuch as the adrenal cortical hormone or hormones appear to have functions other than those dealing with salt metabolism there are no signs or symptoms which can be entirely ascribed to a deficiency *per se* of sodium and/or chloride

Another instance of excessive loss of these two elements is found when there is profuse sweating Experimental sodium chloride deficiency has been studied by McCance (768) who in himself and a group of fellow subjects has described anorexia nausea fatigue a sense of exhaustion and muscle cramps As would be expected there was hemoconcentration All such manifestations of the deficiency disappear following the ingestion of salt and water

Sulfur

Historical The element sulfur has been known from earliest times Deficiency of inorganic sulfur is dwarfed by the importance of deficiencies of the sulfur-containing amino acids methionine and cystine (see page 82) No pointed experiments have been directed at a study of the effects of a deficiency of inorganic sulfur utilizing diets containing varying quantities of the two sulfur amino acids

Biochemical Relationships Sulfur is found in a wide variety of compounds in the organism and is physiologically one of the most important elements For instance it occurs in amino acids (methionine, cystine) hormones (estrogens insulin) a variety of enzymes certain vitamins (thiamine biotin) taurine carbohydrates (chondroitin sulfuric acid) and lipids (sulfatides)

The functions of ingested inorganic sulfur are not at all clear The biochemical relationships of organic or amino acid sulfur compounds are

placed by stratified squamous epithelium In the cornea the initial change is a migration of leukocytes into the substantia propria followed by an ingrowth of capillaries In the early stages of cellular and vascular infiltration the corneal epithelium shows no change, later however, it becomes keratinized The cause or causes of the corneal changes are not clear, but are interesting to speculate upon It is well known that the corneal epithelium is

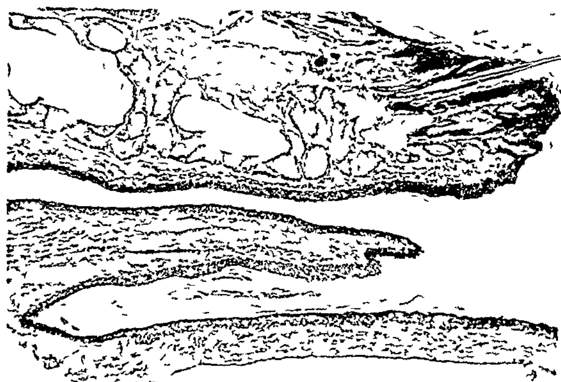


FIGURE 10 Eye Sodium Deficiency (110) Section through anterior segment of the eye of a rat which had been on a sodium deficient diet for 84 days The upper lid can be seen above with tremendously dilated tarsal glands The inner lining epithelium is transformed into a stratified squamous type A similar metaplasia is found in the corneal epithelium In addition blood vessels and leukocytes are found infiltrating the substantia propria of this structure H and E ×100

unique in that it is in contact with an hypertonic environment produced by evaporation of the tears In the sodium depleted animal, the sodium content of the tears is doubtless reduced with consequent drop to iso- or even hypotonicity The corneal epithelium conditioned normally to a hypertonic medium may be extremely sensitive to one which approaches that of normal cells How much of a role absence of the secretions of the meibomian glands plays is fruit for speculation as well

An incidental observation might be mentioned When rats are made hypertensive by reduction of kidney tissue the exclusion of sodium from the diet reduces the elevated blood pressure (111), the reason for this is not understood

Pathological Effects Day and McCollum (114) have reported the preparation of a diet with which to study the effects of phosphorus deficiency, this ration, which contains only 0 017 percent phosphorus has an adequate calcium and vitamin D content When young rats are placed on the diet there is an extreme retardation in growth and the animals appear unkempt and very inactive All the tissues of such animals have been studied by the present writer in association with Day and McCollum (115) Aside from the manifestations of profound inanition the only specific gross or microscopic alterations are found in the skeletal system where extreme rickets is present Due to extensive changes in the ribs, the thorax is greatly deformed and is reduced in capacity Consequently the lungs become extremely atelectatic so that it is quite apparent that respiratory difficulty contributes in large measure to the fatal outcome which occurs after eight or nine weeks The thoracic deformities are similar to those which Park and Howland (116) described a number of years ago in rachitic children Microscopically, the alterations in the bones of phosphorus deficient rats are typical of rickets and are observed after the animals have been on the experimental diet for only one week In the latter stages of the deficiency that is, after the sixth or seventh week the skeletal evidences of active rickets become less conspicuous due to the slowing and virtual cessation of growth, in fact in the end the rickets begins to heal The description of the histological changes will not be detailed here since the pathologic anatomy of rickets is described on page 110

Metabolic studies (114) of phosphorus-deficient animals reveal continuous negative balance of this anion Despite this a significant amount of phosphorus is apparently removed from the bones and redeposited in the soft tissues No appreciable derangements in metabolism or sodium, potassium or magnesium have been noted

The experimental diet of Schneider and Steenbock (113), referred to above contains somewhat more phosphorus calcium and vitamin D than that used by the Johns Hopkins investigators The former workers observed calcium citrate calculi in the kidneys ureters and bladder of their rats (117) Furthermore they concluded that the vitamin D present in the diet reversed the soft tissue vs bone preference for phosphorus in favor of the latter tissue an hypothesis which is of great theoretical interest especially as it relates to the mode of action of vitamin D The only other change which has been described in the phosphorus-deficient rat is parathyroid hyperplasia (45) The enlargement of the glands is not as marked as that encountered in calcium deficiency The experiments which have been performed however are open to question since purified diets have not been employed and parathyroid hyperplasia does not appear to occur under such circumstances (115)

discussed elsewhere (pages 82–101). When inorganic radioactive sulfur is fed to rats, it does not appear in the cystine of the hair or carcass (33); nor can this species utilize elementary sulfur in lieu of cystine or methionine, for when sulfur is fed to animals deficient in these amino acids, virtually all finds its way into the urine where it is excreted as sulfate (34). Finally, if rats are fed colloidal radioactive sulfur, none can be detected in their body protein (35). Such studies make it apparent that inorganic sulfur cannot be utilized to build or replace sulfur-containing amino acids. However, whether small amounts of inorganic sulfur can be synthesized into utilizable compounds of other types remains a question which as yet has not been answered.

Pathological Effects. From the little evidence that is available, it appears unlikely that inorganic sulfur deficiency can occur; the changes which take place when there is a deficiency of the sulfur-containing amino acids are described elsewhere on page 82.

Phosphorus

History. Because of its widespread distribution, phosphorus has been regarded as an essential nutrient for some time. The first pointed experiments were performed in 1918 by Osborne and Mendel (77) who showed that this element is necessary for growth of rats. The experiments of Sherman and Pappenheimer (78) which followed clearly demonstrated the role of phosphorus in the production of rickets. It only remained to show that phosphorus deficiency produced pathological effects when all known nutrients, including vitamin D, were present in the diet, something which was accomplished by Schneider and Steenbock (113) in 1939.

Biochemical Relationships. Approximately three-quarters of the body's store of phosphorus is found in the skeletal system. In addition to this important role in the formation of bone salts, phosphorus is also one of the most important (perhaps the most important) element, excluding carbon, hydrogen, oxygen, nitrogen and sulfur, in physiological processes since it is concerned with the liberation of energy for muscular contraction, secretion by the kidney, et cetera. Its functions are too familiar to require anything but brief mention: in the carbohydrate cycle and in muscle metabolism, in lipoid metabolism (lecithin, cephalin), in protein metabolism (nucleic acids, creatine, ATP, ADP) and as a constituent of certain enzymes (cocarboxylase, flavo-proteins, pyridine nucleotides).

Because of the interrelationships of phosphorus, calcium, vitamin D and the parathyroid hormone, further discussion of phosphorus metabolism will be found in the chapter on vitamin D on page 104.

Chlorine

Historical. The indispensability of chlorine in the diet was first shown by Orent-Keiles, Robinson and McCollum in 1937, when rats are placed on a synthetic low-chloride diet they fail to grow in normal fashion (107).

Biochemical Relationships. In the organism chloride ions occur principally in the extracellular spaces and account for about two-thirds of the 155 milliequivalents of the acidic constituents of extracellular fluid (49). Chloride, of course, is an important secretory product of the gastric mucosa. Strangely enough little else is known of the function of chlorine in the animal organism except for its activation of the salivary enzyme ptyalin.

Gersh (79) employing an histochemical method has described the distribution of chloride in voluntary muscle. Here it is found only in the intercellular spaces, none is present in the muscle fibers themselves.

Pathological Effects. Physiological as well as pathological observations have been made by several groups of investigators on chlorine-deficient animals. The morphological observations are not adequate, however. Using a diet of unknown though low-chloride content Orent-Keiles et al. (107) could detect no changes other than a disturbance in growth, even after rats had been on the experimental regimen for as long as ninety days. Histological studies of such animals were not performed. These observations have been confirmed by other investigators using diets with various concentrations of chloride ions (119, 120). When a diet containing only 0.02 percent chloride is utilized there is a retardation in growth, together with a reduction in the sodium, potassium, and chloride content of the tissues and an increase in calcium and phosphate concentrations. When a similar diet containing only 0.012 percent chloride is employed (121), rats again fail to gain and dramatically exhibit a conservation of chloride, for after the animals have been on the deficient diet for only a few hours the urinary chloride excretion decreases to virtually zero. Compared with controls which excrete 110 to 170 milligrams of chloride per day, the deficient animals excrete only 0.5 to 1.2 mg. per day. In such rats there also is a reduction of serum chloride from 295 milligrams per hundred milliliters to 252 milligrams and an increase in the carbon dioxide combining power from 58.8 to 72.3 volumes percent. However, the manifestations of tetany have not been observed. It would be interesting to augment the chloride deficiency produced by dietary means with that produced by removal of gastric secretions. Cuthbertson and Greenberg (122) have concocted an even lower chloride-deficient diet (2-5 milligrams percent chlorine) and have shown a reduction of the chloride concentration in skin, muscle, liver, kidney, testis, brain, stomach, lungs and

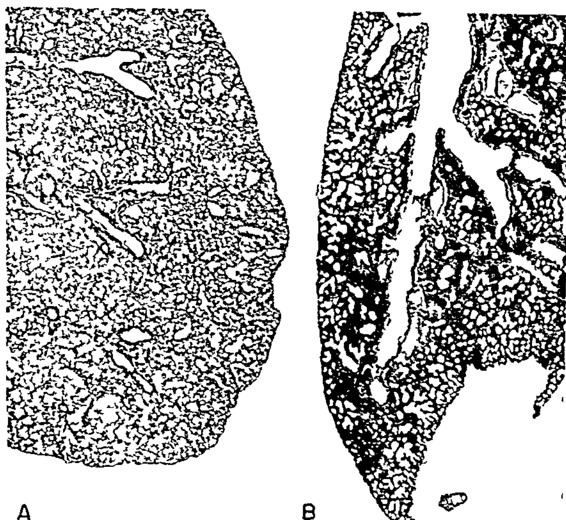


FIGURE 11 A Mechanical Effect of Rickets on the Pulmonary Tissues (116) *A* Normal lung which is aerated save for some atelectasis about the peripheral portion a constant phenomenon following opening of the chest *B* Section of lung from a rat on a phosphorus deficient diet (0 017 percent) for 49 days Due to the extreme decrease in the capacity of the thorax as a result of collapse of the bony cage there is severe atelectasis especially in comparison with *A* which is from a normal control receiving added phosphorus in the deficient diet both H and E, ×16

Phosphorus deficiency has been studied in dogs no specific changes other than severe rickets are found (118)

Phosphorus Deficiency in Man Uncomplicated phosphorus deficiency in man is a subject which is difficult to comment upon since evidence for an accompanying calcium and/or vitamin D deficiency cannot usually be ruled out It is possible that certain instances of lead poisoning in children accompanied by rickets may be examples of conditioned phosphorus deficiency as a result of the formation of insoluble compounds in the intestine (386)

reserve, hyperexcitability, and convulsions all of which may be prevented by the administration of chloride ions

Iron

Historical Iron salts are said to have been used by Sydenham during the eighteenth century for the treatment of chlorosis The full significance of iron for the organism was realized when this element was demonstrated to be an essential component of hemoglobin

Biochemical Relationships Iron is absorbed in the upper portion of the small intestine (123) Species differences appear to determine whether ferrous or ferric salts are absorbed more readily (124) A most important factor in absorption appears to be the state of the organism's store of this element, if the tissues are depleted iron is rapidly absorbed from the intestinal tract, on the other hand if sufficient quantities are present there is little absorption (123) Clinical studies would seem to indicate that the pH of the gastric juice plays a role in the absorption of iron for in females exhibiting the syndrome of idiopathic hypochromic anemia there is achlorhydria (125) It has further been shown that iron is absorbed more rapidly from acid than from alkaline media Little excretion of the element takes place by either the alimentary tract or kidneys (126) so that iron has been called a one way substance (127)

Iron occurs in three main portions as a part of hemoglobin as tissue iron and as storage iron Keilin (128) has divided the first two types of iron compounds, which have great biological importance, into oxygen carriers and oxidizing catalysts, both of which are iron-porphyrin-protein complexes To the oxygen carriers belong hemoglobin and myohemoglobin, among the oxidizing catalysts are included the cytochromes catalase, and peroxidase The third form of iron so-called storage iron' occurs mainly as hemosiderin which is thought to be composed of an organic material impregnated with ferric oxide (129)

Iron may be demonstrated in tissues by suitable stains Hemosiderin exhibits the familiar Prussian Blue reaction Organic iron, presumably that composing iron-porphyrin-protein complexes, can be demonstrated by treating the section with hot acid alcohol and then staining with ferrocyanide (130)

Pathologic Effects Despite the large literature that has been accumulated on iron deficiency anemias in the human virtually no studies have been reported on pathological changes occurring in the tissues of humans or of experimental animals Hematological data have been reported in rats (131)

total carcass The chloride content of the heart and spleen appears to be increased In such animals an interesting pathological change has been described in the kidneys, where lesions develop as early as one month following the institution of the low-chloride regimen (788) The pathogenesis of the changes in the kidney have been interpreted as follows due to the



FIGURE 1[illegible] Kidney Chlorine Deficiency (788) Renal tissue from a rat on a chloride deficient ([illegible] mgm percent) diet for [illegible]6 days Note dilated tubules lined by flat epithelium imbedded in a connective tissue stroma The lumens of the tubules contain calcified casts which would eventually have led to obstruction and dilatation of the proximal portion of the tubule H and E ×150 (Courtesy of Dr F Lowenhaupt and Dr D M Greenberg)

alkaline urine and possible elevation in phosphate concentration in the convoluted and collecting tubules there is a precipitation of calcium salts in these structures This leads to obstruction of the lumens and initiates a reaction in the tubules and peritubular tissues Many of the lumens may become obstructed which leads to a shell filled with fluid consisting of a much thinned cortex with pelvic epithelium forming folds separated in part from the compressed cortical zone' No lesions were found in the other tissues of these animals An arrest of spermatogenesis is doubtless the result of inanition

Chloride Deficiency in Man The role of chloride alone in sodium chloride deficiency in man is not clear However large amounts of chloride ions may be lost under another circumstance pyloric obstruction with resulting gastric tetany (784) Here there is a great increase in the alkali

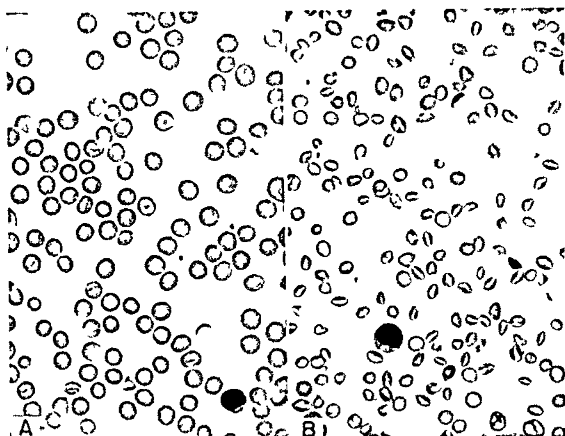


FIGURE 1 Blood Iron Deficiency (734) *A* Smear taken before young rat was placed on a regimen of whole milk supplemented with copper *B* Smear at time of sacrifice forty days later Note microcytosis achromia as well as poikilocytosis The blood showed the following (compare with normal values on page 48) RBC 4 [illegible]90 000 Hb 3 7 gm MCV 44 cu μ MCHC 19 percent MCH 8 6 μμ Wright stain ×600

corpuscular volume and the total number of cells when iron therapy is instituted Several clinical types of anemia should be mentioned (776) Chlorosis is an example of anemia which results from inadequate intake of iron this syndrome which used to occur primarily in young women is now uncommon As has already been mentioned achlorhydria may affect the absorption of iron (125) so that in many cases of hypochromic anemia especially in women it is common to find a decrease or absence of hydrochloric acid in the gastric contents So too when the passage of the intestinal contents through the intestinal tract is rapid iron may be poorly absorbed, hypochromic anemia is therefore seen in cases of chronic diarrhea During pregnancy there is an increased requirement for iron in order to supply the fetus, during gestation therefore hypochromic anemia may be encountered The rapid growth period of childhood is another critical stage when supplies of iron must be adequate Chronic blood loss usually coupled with insufficient iron intake is a most important factor in the production of hypochromic anemia hemorrhage from the gastro-intestinal tract because of a variety of

rabbits (132) and swine (653) It is agreed that a microcytic hypochromic anemia develops when iron is withheld from the diet of growing animals whose rations are adequate in all other respects, including the copper content When rats are placed on a milk diet allowed to become anemic and then treated with copper Smith and Medlicott (131) find the differences in the blood picture as recorded in the following table

Table III

	Normal	Iron Deficient
Hemoglobin (gms 100 ml)	14.91	3.37
Red blood cells (cmm)	7,421,000	4,2[illegible] 000
Mean corpuscular volume (cu μ)	60	34.6
Mean corpuscular hemoglobin conc (%)	33.3	23.0
Mean corpuscular hemoglobin (γγ)	20.2	8.0
Reticulocytes (%)	3.4	23

Smears of the blood from iron-deficient animals reveal rather marked microcytosis and achromia Tiny little cells are seen which are pale and *sometimes are very difficult to recognize There is also basophilia of many* of the more normal-sized cells (734)

In iron-deficient animals the serum iron concentration is reduced In swine for instance average values of 48.0 gamma percent have been reported in deficient animals, the average concentration in controls is 142.7 gamma *percent (653) The iron content of the tissues of rats is also reduced when* dietary intake of iron is restricted (136), the teeth of such deficient rats have a lower iron concentration than that of controls, there is also loss of the yellow pigment of the growing incisor which is not surprising since this pigment is an iron containing complex (616)

In contrast to copper deficiency (page 51) the cytochrome oxidase of the bone marrow of iron-deficient animals is normal or sometimes even elevated (134) though how much of a role differences in cellularity of the marrow play in the results of such determinations should be taken into account such measurements of the cytochrome oxidase activity should therefore be controlled by histological observations

Iron Deficiency in Man Iron deficiency in the human particularly in women and children, is probably more common than has been realized One may postulate and prove that there are several general factors one or more of which may result in the development of iron deficiency The following are therefore of importance in the pathogenesis of iron deficiency anemia inadequate intake such as has just been described in the experimental animal, defective absorption increased requirements and excessive loss (776)

Iron deficiency *anemia is characterized by* microcytosis and hypochromia there is a prompt reticulocyte response and return to normal of the mean

confused the picture in other similar experiments Copper occurs as a constituent of certain oxygen carriers for instance the hemocyanins of lower organisms (128) In addition certain oxidizing catalysts such as polyphenol oxidase and ascorbic acid oxidase contain copper The significance of a copper containing compound hemocuprein which Keilin has obtained from mammalian red blood cells is not clear The element has also been found in

Table IV

	Normal	Copper deficient
Hemoglobin (gm ml)	14.91	[illegible]
Red blood cells (cmm)	7,471,000	2,776,000
Mean corpuscular vol (cu μ)	60	[illegible]
Mean corpuscular hgb con (%)	33.3	26.8
Mean corpuscular hgb (γγ)	20.2	13.7
Reticulocytes (% per 100 RBC)	3.4	7.8

the elementary bodies of vaccinia virus, here again its role has not been clearly elucidated (621)

Methods for the determination of copper in tissue sections have been described but as yet have not been applied to animals on copper-deficient diets (622)

Pathological Effects Knowledge of the pathological changes which accompany copper deficiency has been derived from several sources The experimental studies of Hart et al (617) which have been confirmed by others first showed that an anemia develops in animals whose diet is deficient in copper even though adequate amounts of iron are present Achromotrichia has also been described in animals on copper deficient diets Workers in the field of veterinary medicine later showed that characteristic symptoms occurring in both sheep and cattle probably resulted from the low copper content of certain pasturages and that the inclusion of copper salts in the food of such animals prevented the appearance of the specific manifestations of the disease

The anemia which develops in the copper deficient rat (131) and rabbit (132) tends to be somewhat microcytic and hypochromic in character The following table provides hematological values which have been recorded by Scott and Medlicott (131) on rats rendered anemic by a milk diet and then given insufficient dietary copper but adequate iron

No reports of any histological observations on laboratory animals deficient in copper have appeared Certain chemical studies are of interest, however The cytochrome oxidase activities of liver myocardium and bone marrow are decreased in copper deficient rats (133 134) Such observations are probably important because a close relationship has been demonstrated between the cytochrome oxidase activity of bone marrow and its ability to form hemo-

lesions including hookworm infestation (778) and menstruation play extremely important roles in this connection. In all of the clinical syndromes briefly alluded to treatment with iron evokes a prompt reticulocyte response and return of the blood picture to normal. Of course, in order to have the red blood and hemoglobin concentration remain at the normal limit, the underlying causative mechanism must be eradicated.

Since virtually no cases of clinical hypochromic anemia die, the pathological changes in the tissues, if any, are not clearly understood. The bone marrow is said to exhibit normoblastic hyperplasia (776). In view of the scant pathological manifestations of iron deficiency in experimental animals it is worthwhile to mention certain other changes which are encountered in iron deficient anemias in the human. Sore tongue and sore mouth similar to those encountered in nicotinic acid and riboflavin deficiencies (pages 166 and 158) have been described and respond to therapy with iron (777). In addition there may be extreme dysphagia (the Plummer-Vinson Syndrome) (776). Another interesting finding is the presence of koilonychia or longitudinal ridging and flattening of the fingernails which may be even concave instead of convex.

Copper

Historical. The indispensability of copper for the animal organism was announced in 1928 by Hart, Steenbock, Waddell and Elvehjem (617). These investigators showed that rats develop a severe anemia on a milk diet and that this anemia does not respond to the administration of iron but can be relieved when the milk diet is supplemented with copper as well.

Biochemical Relationships. Copper is widely distributed in the animal organism. The central nervous system is richer in its content of this element than any other tissue except liver (618). The following concentrations of copper in mg. per kilo. of dry weight of certain human tissues have been reported: liver, 40.2; cerebellum, 28.8; cerebrum, 18.1; kidney, 14.1; heart, 13.4; pancreas, 8.7; and muscle, 6.4.

Copper is actively concerned with hematopoiesis although its precise physiological role in this process is not at all clear. It has been shown, however, that copper has little, if any, effect on iron absorption (619). Copper seems to be necessary for both hemoglobin synthesis as well as erythropoiesis (620), for when this element is administered to rats on a milk diet to which no iron has been added, a rise in red blood cells occurs, although there is no marked increase in hemoglobin. It, of course, can be argued that the copper salts contained traces of iron; the use of impure supplements has

hind limbs, severe incoordination and in some instances blindness As mentioned above the malady is seen only in newborn or very young lambs, in some flocks the incidence has been as high as ninety percent of all the animals born The pathological changes in lambs exhibiting manifestations of swayback were reported by Innes in 1934 (144) before the relationship of copper

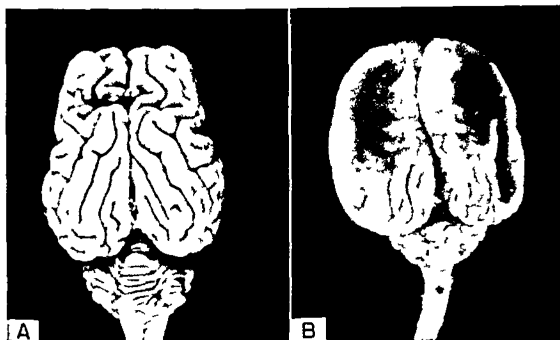


FIGURE 14 Brain Copper Deficiency (145) Gross appearance of brain *A* from normal newborn lamb and *B* from an animal dying three days after birth with signs of "swayback" Note collapse of cerebral hemispheres and shallow convolutions (Courtesy of Dr J R M Innes and *The Journal of Comparative Pathology and Therapeutics*)

deficiency to the syndrome had been fully elucidated Innes has since extended his studies to the nervous tissues of a large group of lambs affected with the disease and has carefully described the extraordinary changes (145)

The external appearance of such a brain is quite extraordinary since the entire size is smaller than that of a newborn lamb and in addition there is shrinkage and depression of the cerebral hemispheres due to the obvious loss of substance beneath

Grossly the lesions vary from small areas of porencephaly in the white matter of the central hemispheres to those in which the central white matter was restricted in most cases to a grossly degenerated *centrum ovale*, to a wasted *corpus collosum* and *septum pellucidum* and to the internal capsule The cerebral gray matter was relatively well preserved, but formed only a thin shell around the degenerated white matter of the cavity '

Microscopically in the white matter of the cerebral hemisphere there is symmetric diffuse demyelinization The process varies from foci of micro-

globin and erythrocytes. That copper acts somehow in hematopoiesis is further indirectly indicated by the ready entrance of radioactive Cu_{29}^{64} into the bone marrow (135) and by the fact that copper stimulates erythropoiesis, for when the element is administered to animals on a milk diet, there is a rise in erythrocytes without a concomitant elevation of hemoglobin (620). Copper deficiency may produce changes in other organs, since rats on low copper diet succumb before any well-marked anemia appears and at autopsy the spleen and liver are said to be enlarged (136). Histological examinations of the tissues of copper deficient animals are, therefore, much to be desired.

In addition to its importance for the formation of red blood cells, copper also plays a role in the pigmentation of hair. Keil and Nelson (137) first demonstrated that the fur of black-coated rats becomes gray after such animals are placed on a copper-deficient diet. With the discovery of other achromotrichia factors, such as pantothenic acid and para-aminobenzoic acid, the relationship of copper to graying of hair became somewhat confused. Henderson et al. (138) have clarified the situation, however, by showing that the achromotrichia produced by copper deficiency is not affected by large amounts of pantothenic acid; these investigators also point out that the fur in animals deficient in copper has a brownish color, contrary to the silvery-gray which develops when pantothenic acid is lacking. No relationship of copper to the lack of pigmentation of hair of mice deficient in para-aminobenzoic acid has been demonstrated (738).

Studies of spontaneous or endemic copper deficiency have been reported in sheep in Australia (139) and England (140) and in cattle from the former region. The disease in sheep has been called "enzootic ataxia" or "swayback." Although the importance of copper to this malady is considered equivocal by some until experimental studies are carried out with purified diets, it would seem that swayback must be due to a deficiency, a conditioned deficiency perhaps, of copper. It seems thoroughly well established that in Australia the syndrome occurs only in those pasturages whose copper content is low, although this is not confirmed by English investigators. Blood copper studies of pregnant ewes have generally revealed decreased concentrations of this element (141). As was first shown by Bennetts and Chapman (139) in Australia and subsequently confirmed by Dunlop et al. (140) in England, the incidence of swayback can be greatly reduced or even eliminated by the administration of copper (142). Further studies have shown that other trace elements, such as iron and cobalt, are not effective and that treatment with copper raises the blood concentration of this element in pregnant ewes (142). The most important changes have been found in the newborn lamb, in which one of the most interesting manifestations of a deficiency in a single nutrient is the relation of copper to lesions in the nervous tissue of animals born to copper deficient ewes. The signs consist of spastic paralysis, especially of the

man His photographs which he has so kindly allowed us to reproduce confirm this similarity

In adult cattle a syndrome called falling disease" or 'sudden death' has been described to be dependent upon a deficiency of copper since the administration of this element leads to a reduction in the incidence of the malady



FIGURE 16 Brain Copper Deficiency (145) *A* Section from occipital pole to show loss of myelin with cavity formation at several points Weigert Pal x4 *B* Higher power of neurons in red nucleus two of whose cells show chromatolysis Nissl Orange G method, x400 (Courtesy of Dr J R M Innes and *The Journal of Comparative Pathology and Therapeutics*)

(146) The syndrome is characterized by anemia loss of weight, and sudden death The reduction in hemoglobin is not severe however At autopsy there is extensive hemosiderosis of the spleen liver and kidney Lesions have been described in the kidney and myocardium In the former tissue degeneration of glomeruli is said to be prominent The tubular epthelium shows cloudy swelling, the lumens are filled with detritus From the description it is difficult to make any decision as to the exact nature or pathogenesis of these renal changes The heart of affected cows shows extreme fibrosis, which has been interpreted to result from atrophy of the muscle fibers due to long-standing anoxemia (starvation atrophy) Apparently neurological lesions are not prominent

In summary, copper deficiency in experimental animals leads to anemia, endemic copper deficiency in sheep is associated with widespread myelin degeneration in their offspring

Copper Deficiency in Man It is unlikely that copper deficiency occurs at all in man Although evidence has been brought forward that certain cases of anemia in childhood may be benefitted more when both iron and copper therapy are used the evidence for this is not considered conclusive by others (127)

scopic size to a virtual absence of the myelin, which can be observed grossly in the severest cases, such as those described and illustrated above In the areas of demyelinization the axis cylinders disappear, moderate glial proliferation is found in and about such areas Destruction of myelin has not been encountered in the midbrain, cerebellum or brain stem, but as might

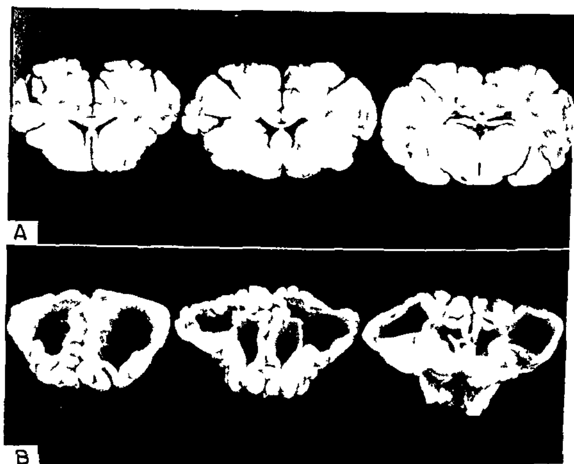


FIGURE 15 Brain Copper Deficiency (145) Coronal sections *A* through brain of normal two day old lamb and *B* animal dying five days after birth with characteristic signs of swayback Note extreme destruction of white matter with cavity formation and wasting of the corpus callosum The gray matter is relatively well preserved The ventricles of the affected brain appear to be dilated (Courtesy of Dr J R M Innes and *The Journal of Comparative Pathology and Therapeutics*)

be expected, degeneration of the descending tracts has been observed in the spinal cord In those lambs in which clinical evidence for the presence of the disease was slight, the neurons usually show no change while in those exhibiting more severe symptoms degenerated cells are found A constant site of degeneration is the red nuclei No inflammatory reaction is present in the tissues about blood vessels save the occurrence of mononuclear phagocytes filled with lipoid Innes has called attention to the similarity of the pathological manifestations of this condition in lambs to Schilder s Disease in

similar studies were being pushed in the southern part of that continent. In 1935 Marston (156) reported in sheep a wasting disease with an associated anemia. The administration of cobalt leads to a marked gain in weight together with rises in hemoglobin and red blood cell count (157). This malady, 'coast disease,' as it has been called, is in some instances accompanied by ataxia.

Utilizing the Marchi method, which is an unreliable one, degeneration and demyelinization of certain tracts in the spinal cord have been demonstrated (158). In affected animals histological examination of the tissues reveals increased deposits of hemosiderin in the liver, spleen, pancreas, and kidney, and on chemical analysis iron is found in large quantities in these tissues (160). It was demonstrated that "coast disease" is a syndrome caused by a deficiency of both cobalt and copper (159). The neurological lesions may then be related to those described in uncomplicated copper deficiency of sheep (page 52).

A disease similar to "enzootic marasmus" has been reported in Florida (161). The muscles of affected calves are pale and there is a great loss of body fat. Degenerative changes are found in the heart and the spleen is said to be shrunken, the liver fatty. More precise pathological studies of animals such as these and in addition those from affected pasturages in Australia are greatly to be desired.

In summary, cobalt has a questionable effect on blood formation in experimental animals and a marked effect on hematopoiesis in farm animals.

Cobalt Deficiency in Man. There is no evidence of cobalt deficiency in man and it is unlikely that an experimental deficiency could ever be produced.

Manganese

Historical. The indispensability of manganese for the animal organism was shown simultaneously in 1931 by Orent and McCollum (163) and Kemmerer, Elvehjem and Hart (162).

Biochemical Relationships. Manganese is widely distributed in tissues, although there are variations from one organ to another; the element is particularly abundant in liver (164). The only biological functions of manganese discovered thus far are its relationships to the activity of certain enzymes. Manganese, like magnesium, is necessary for the activation of phosphatase (165); a more important role is its actual presence in the enzyme, arginase, which is utilized in the formation of urea (166).

Pathological Effects. Unfortunately experiments dealing with manganese deficiency have been almost entirely restricted to macroscopic or physio-

Cobalt

Historical Cobalt deficiency has not yet been produced in experimental animals The indispensibility of cobalt in the nutrition of sheep and cattle, however, was demonstrated in Australia by Filmer and Underwood during the period from 1933 to 1935

Biochemical Relationships The relationship of cobalt to hematopoiesis was shown a number of years ago by the Waltners (147), who reported that polycythemia can be produced in rats when this element is administered The cause for the erythrocytosis is not clear Although it had been suggested that cobalt might interfere with the respiration of the bone marrow no evidence has been brought forward for this assumption (148) In rats to which cobalt is administered the average hemoglobin values may rise to 20 5 gm The spleens of such animals are said to be enlarged, microscopically there is increased erythropoietic activity in the spleen, bone marrow, and to a lesser extent in the liver (149)

Pathological Effects Efforts to produce cobalt deficiency in laboratory animals have not been successful Workers at the University of Wisconsin have prepared diets containing only 6 micrograms of cobalt per kilo (150) At this low dietary level no effects appear in rats However, when dogs are placed on a similar ration and are rendered anemic by bleeding the administration of cobalt seems to stimulate hematopoiesis in some of the animals (151) This approach to the subject would seem to warrant further experiment

The story of cobalt deficiency in farm animals is quite different from that which has been encountered in the laboratory and is one of the triumphs in veterinary nutrition In 1933 Filmer (152) called attention to a disease of sheep and cattle in western Australia The syndrome which he called 'enzootic marasmus had been recognized for some time and is characterized by severe emaciation and wasting together with anemia Microscopically the liver is fatty and contains hemosiderin, the spleen and kidney also contain large quantities of iron pigment So too upon chemical analysis large amounts of iron are found in the tissues of both sheep and calves in comparison with those of normal animals (153) Although it was first shown that iron salts and liver are effective in curing the disease (152) subsequent work has revealed that impurities in the iron-containing limonite salts which were used are the active agents (154 155) and that of these cobalt is the specific material in question (155) The element is found in insufficient quantities in the pasturages upon which the affected animals feed

While these investigations were being carried out in western Australia

More complete studies of the osseous changes are necessary before final conclusions can be drawn in both the rat and the rabbit.

Sterility of male rats on manganese-low diet has been a constant finding. Microscopic examination reveals absence of spermatogenesis (170), but it is not clear whether this change is a result of inanition or not, a point which has not been specifically investigated.

The liver tissues of manganese-deficient rats exhibit a decrease in arginase activity (169, 170). For instance, 1.051 milligrams of urea may be formed by one milligram of dry normal liver at 28° C, while only 0.561 milligrams of urea are formed by the same weight of liver from the manganese-deficient rat (169). Addition of manganese restores the arginase activity to normal; despite this change *in vitro*, no evidence of abnormal nitrogen metabolism can be demonstrated *in vivo*.

In summary, manganese deficiency in experimental animals leads to disturbance of the growth of young born of deficient mothers. Many die and the few which survive have changes in the bones and develop ataxia.

Manganese Deficiency in Man. There is no evidence for manganese deficiency in man, nor is it likely that such a deficiency can ever be produced experimentally.

Zinc

Historical. The indispensability of zinc in the diet of the rat was first shown by Todd, Elvehjem and Hart (174) in 1934; the experimental animals exhibited a disturbance of growth and alopecia.

Biochemical Relationships. Little is known of the role of zinc in biologic processes except for its occurrence in several important enzymes. Highly purified preparations of carbonic anhydrase contain about 0.3 percent zinc (175). This enzyme, which is so important in the reaction $H_2CO_3 \rightarrow CO_2 + H_2O$, is found in red blood cells and other tissues. Zinc also occurs in association with uricase (176), an enzyme which catalyzes the reaction: uric acid → allantoin. Phosphatase is another enzyme which contains zinc (177). In the presence of amino acids zinc markedly activates intestinal phosphatase but has less effect on phosphatase from bone and kidney (178).

Zinc is a constant constituent of the tissues of the rat, the cat and man. For typical values which have been obtained the papers of Lutz (179) and Drinker (180) should be consulted. Studies utilizing radioactive Zn^{65} in rats have shown that the element is mainly excreted into the gastrointestinal tract by the pancreatic juice. It disappears rapidly from plasma and appears early in red blood cells and liver. The most active turnover is found in the liver, pancreas, kidney and pituitary. The least active turnover is in the red blood cells, brain, skeletal muscles and skin (181, 182, 183).

logical observations Since 1931, when Orent and McCollum (163) and Kemmerer et al (162) demonstrated the essentiality of manganese, a number of other reports have been published, particularly by investigators at Johns Hopkins and the University of Wisconsin

In the initial experiments of the former group (163), growth of the experimental rats is normal The estrus cycles of the female are likewise normal (167) and when such animals are mated with normal males the usual number of young are produced However, the females fail to nourish either their own or foster young When young from manganese-deficient females are placed with normal, lactating animals a few are raised, all such young are undersized Divergent results have been obtained by Daniels and Everson (168) who employed a somewhat different diet from that used by the Baltimore workers Almost one-half of the young born to manganese-deficient females on this diet are born dead or die within a few hours after birth Such manganese-deficient females are able to nourish and rear foster young while only a few of their deficient young can be raised by foster mothers It is thus apparent that the fault is in the young and not in the mother This hypothesis has been confirmed by Shils and McCollum (169), who note no disturbance in the estrous cycle of the rat which the University of Wisconsin group had noted in mice (162)

Using a somewhat different diet Boyer et al (170) have shown that manganese is necessary for growth, an observation which has been confirmed by Shils and McCollum (169) The latter investigators have also called attention to another specific feature of the manganese-deficiency syndrome Some of the young of manganese-deficient females exhibit ataxia incoordination and loss of equilibrium they tend to fall over and have difficulty in righting themselves The underlying cause of these phenomena has not yet been elucidated

Changes in the bones have been noted by several observers Barnes et al (171) found roentgenographically that the tibiae of two manganese-deficient rats were shorter than normal and that the epiphysis of the proximal end of the tibia was narrower than normal Shils and McCollum (169) have noted shortening and bowing of the forelegs in the same species Further evidence of the role of manganese in the growth of bone in the rat indicates that when this element is deficient there is a decrease in length and density, as well as in the breaking strength of the bone (173) Phosphatase activity is also reduced In accordance with the observations of others no differences have been found in the percent of ash or calcium phosphorus and manganese contents In manganese-deficient rabbits Smith et al (172) have described bowing of the forelegs which by x-ray examination appears to be rarified It should be pointed out that their diet contains no vitamin D and that the control bones appears in the microphotograph to have an excess of osteoid



FIGURE 18 Skin Zinc Deficiency (185) There is a crust of partially keratinized cells overlying a layer of thickened epithelium which shows acanthosis There is atrophy of the hair follicles with persistence of the sebaceous glands in fact these structures are hypertrophied in comparison with the normal This animal had been on a zinc deficient diet for 74 days H and E x150

nuclei of such cells become pyknotic In advanced cases the skin is covered by a crust of keratinized debris bacteria and leukocytes and the corium underlying such areas is hyperemic and infiltrated with leukocytes Accompanying these changes there is atrophy of the hair follicles, which ultimately disappear leaving only a few mononuclear cells to mark where they had been The sebaceous glands remain intact and even late in the deficiency the cells making up these structures are larger than those of the controls Skin from the tail, ears and paws shows no abnormality

Extensive changes are found in the esophagus of zinc-deficient animals where alterations consisting of an increase in thickness of the epithelial lining together with the appearance of large partially keratinized cells on the surface are found The normal esophageal epithelium consists of a basal layer of cells some of which exhibit mitotic figures Above this there are several layers of larger cells with large clear nuclei whose cell borders are indistinct Above these an abrupt transition to a keratinized layer occurs The basal cells of the zinc-deficient rat are more numerous and closely packed, the overlying stratum is six to eight cells thick Along the innermost edge the



FIGURE 17 Skin. Normal Note epithelium and underlying corium with hair follicles and sebaceous glands The epithelium is several cells in thickness. There is some keratinization although this is not marked. The sebaceous glands are not very large H and E x 0

Pathological Effects Todd et al (174) employing a diet which contained only 1 6 parts zinc per million noted a disturbance in growth and alopecia both of which manifestations could be prevented by supplementing the diet with about 100 micrograms of zinc each day

Day and McCollum (184) have devised a diet which furnishes only one to four micrograms of zinc daily Zinc is removed by extraction of the dietary components with diphenylthiocarbazone a laborious process The control diet furnishes 15 mg of zinc daily When young rats are placed on such a zinc-deficient regimen they cease gaining weight in two to three weeks Death may occur as early as the thirty-third day of the deficiency Thinning of the hair becomes apparent after the third week and is followed by alopecia over portions of the dorsum of the body The denuded areas are roughened and scaly The histological effects of zinc deficiency were studied by the present writer in association with Day and McCollum (185) Microscopically the skin shows hyperkeratinization and acanthosis The epithelium increases from the normal of three to four cells in thickness to eight or ten cell layers and on the surface an increased number of completely or partially keratinized cells is found No appreciable increase in mitotic activity of the basal cells can be discerned although quantitative measurements have not been performed Clear spaces appear in the cytoplasm of many cells and the

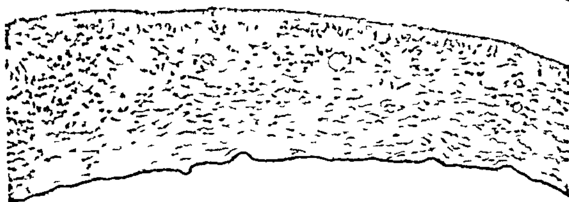


FIGURE 20 Cornea Zinc Deficiency (185) Cornea from zinc deficient animal which had been on experimental diet for 74 days Note several large vessels together with infiltration with leukocytes There is also some thickening of the epithelium The corneal vascularization and leukocytic infiltration is similar to that which has been described in other nutritional deficiencies in the rat H and E, ×60

Zinc deficiency has also been produced in mice (186), which develop alopecia of the shoulders, back of neck and part of the face

Studies of the activity of certain enzymes with which zinc is related are interesting though negative Measurements of the carbonic anhydrase activity of the red blood cells from zinc-deficient animals have shown no change from normal (184 187) Although no decrease in the uricase activity of the liver has been demonstrated in zinc-deficient rats such depleted animals do have an increased concentration of uric acid in the blood (188) Reduction in the activity of the alkaline phosphatase of the blood has been found in zinc-depleted animals (184), no abnormalities in carbohydrate metabolism have been detected (189) nor do such animals show any decreased activity of certain pancreatic enzymes (proteinase amylase) (190)

In summary, experimental zinc deficiency leads to lesions of the skin, esophagus and possibly the cornea Certain metabolic abnormalities have been discussed More chronic studies of zinc deficiency should be undertaken

Zinc Deficiency in Man It is highly unprobable that zinc deficiency ever occurs in man in fact it would require superhuman efforts to concoct a diet with which to study this deficiency in experimental subjects since this element is so widespread in foods

Iodine

Historical Although the existence of iodine had been recognized for many years and this element had even been used from time to time therapeutically its importance for the organism was not clearly demonstrated



FIGURE 19 Zinc Deficiency (185) *A* Normal esophagus *B* Esophagus from rat which had been placed on a zinc deficient diet for 74 days Note increase in thickness of the lining epithelium In comparison with normal there is extensive parakeratosis together with an increase in thickness of the basal cell layer H and E x50

nuclei become pyknotic but do not disappear in normal fashion so that there is a zone of partially keratinized cells ten or twelve cells in thickness The submucosa is normal Similar alterations found in foci on the posterior portions of the tongue and the posterior roof of the mouth, the fore stomach is not involved The change is interpreted as being due to either a retardation in normal keratinization or an increased proliferation of cells The lesion is unique among the many which have been produced by various dietary deficiencies

In two zinc-deficient animals vascularization of the cornea has been observed This consists of an ingrowth of capillaries into the tunica propria and infiltration of this tissue by leukocytes There is no keratinization of the epithelium and the lacrimal glands are normal No other specific changes are found in other tissues of such animals save those which must be ascribed to inanition

Studies of the zinc content of the bones of these animals reveal a reduction in the deficient rats to 94 7 micrograms per gram of ash as compared with the value of 236 6 micrograms in the controls (184)

b) Increased production
c) Decreased need—Starvation hot environment

II Increased—Hyperplastic gland with or without physiological hyperthyroidism

A Primary pituitary hyperfunction ( )
B Injection of thyrotrophic hormone (200)
C Decreased thyroxin in the blood due to
a) Increased utilization—cold poisons (200 201)
b) Removal of thyroid tissue
c) Interference with function
1 Iodine deficiency (201 202 203 204 205)
2 Interference with removal of inorganic iodine from blood stream
3 Interference with reaction inorganic iodine → organic iodine compounds (196)
4 Interference with release of hormone into blood stream (197)

Pathological Effects Studies of iodine deficiency have been intimately concerned with the problem of goiter a term which unfortunately is much misunderstood Goiter has come to mean an enlargement or increase in weight of the thyroid gland Two main types of goiter are observed one due to an abnormal deposition of colloid material in the follicles of the gland so-called colloid goiter and a second due to hyperplasia of the epithelium of the follicles so-called exophthalmic goiter hyperplastic goiter etc It is unfortunate that the term goiter is so frequently used without qualifying it or determining whether such a thyroid is enlarged because of excessive colloid deposition because of epithelial hyperplasia or some other reason

Modern studies of goiter began when Halsted (207) extirpated almost all of the glandular tissue from dogs early in pregnancy and found thyroid enlargement in their off-spring It was soon demonstrated that such congenital goiters could be prevented by the administration of potassium iodide to the female during gestation (208) Further interest was aroused when the administration of iodine to certain pregnant sows prevented the birth of hairless cretin-like off-spring (209) Such experiments lent support to the growing idea that iodine deficiency and goiter are closely related Experimental goiter on the basis of iodine deficiency has been difficult to realize however for any experiment which is designed to study thyroid structure in relation to iodine deficiency must take into account such diverse factors as environmental temperature (200) calcium content of the diet (203) effects of other chemical substances (198 206) as well as many other factors

until 1896 when Baumann (193) found high concentrations of iodine in thyroid tissue. A further clarification of its physiological role awaited the demonstration by Kendall thirty years later that purified thyroxin contains about sixty percent iodine (640).

Biological Relationships. Ingested inorganic iodine compounds are absorbed, find their way into the blood stream, and are immediately removed by the thyroid gland. The thyroid epithelium has an enormous capacity for the prompt withdrawal of relatively large quantities of circulating inorganic iodide which is then rapidly transformed into the organic forms—diiodotyrosine and thyroxine. *In vitro* and *in vivo* studies utilizing radioactive I,^{131} indicate that there is a step-wise mechanism in the formation of thyroid hormone. First, the cells remove inorganic iodide from the medium or blood, next radio-diiodotyrosine and radio thyroxine are formed and lastly an active principle is liberated into the blood stream or colloid. Such conclusions are based on the inactivation of these processes by certain poisons (194, 195, 196, 197).

Before discussing the pathological effects resulting from relative or absolute deficiency, mention should be made of some current concepts of thyroid physiology and of the various factors which affect the gland, for all must be borne in mind when any deficiency of this element is being considered. That there is a reciprocal relationship between the pituitary and thyroid glands seems well established. When, for some reason, the concentration of thyroid hormone in the blood falls, there is an increased production of pituitary thyrotrophic hormone and *vice versa*. Since the hypophysis is thought to be the controlling factor, two groups of possible conditions both based on pituitary activity may be considered: (a) those in which the thyrotrophic stimulus is absent or reduced, and (b) those in which this stimulus is increased. When the former conditions prevail, thyroid hypoplasia (sometimes colloid-like goiter in animals) is to be expected, while thyroid hyperplasia will usually occur following the latter. Thyroid hyperplasia does not always imply clinical hyperthyroidism with an increased basal metabolic rate. On the contrary, it is important to realize that the basal metabolic rate may be elevated, normal or even depressed in the presence of morphologically hyperactive thyroid tissue. The following classification summarizes some of the present knowledge of the various known and hypothetical factors which may lead to the conditions briefly alluded to above.

Pituitary Stimulus is

I. Absent or Reduced—Hypoplastic gland, sometimes colloid like goiter
   A. Hypophysectomy (198)
   B. Increased blood concentration of thyroxine due to
      1) Increased administration (199)

Depending of course on the composition of the diet and its iodine content the initial alteration is found in the epithelium The cells change from their flat or cuboidal form into columnar structures, intra-cellular vacuoles have been described No pointed study has been made of the size of the nuclei or of any other cellular constituent Cellular hyperplasia leads to infolding and partial obliteration of the lumen of the follicles accompanied by a great reduction in colloid content Necrosis of the epithelium has likewise been observed, and is thought to be related to the iodine content of the ration, the lower the iodine concentration the more extensive the necrosis In addition to these morphological changes the gland increases in weight and appears to develop a greater vascularity No lymphocytic infiltrations which are so characteristic of the hyperplastic gland in the human have been described in experimental animals It must be emphasized that the morphological picture thus far produced by experimental iodine deficiency is that of hyperplasia not colloid goiter at least as the latter is seen in man It may be that the experimental periods have not yet been long enough to produce this latter change from the experiments which have just been epitomized it is obvious that more studies over longer periods are greatly needed

Iodine Deficiency in Man Following the early experiments on the role of iodine in the production of goiter in animals it soon became clear that there was a reciprocal relationship between iodine concentration in soil and drinking water and the incidence of goiter in the human The epidemiology of goiter and the results of iodine therapy on the incidence of spontaneous goiter in man have been summarized by McClendon (211) However there is evidence which indicates that the problem is not so simple as many would believe When one realizes that endemic goiter waxes and wanes and that epidemics of goiter have been described it is clear that other factors definitely play a role So too goiter does not seem to have been as prominent during the early exploration of America as it now is These and other observations which indicate only a few of the complexities of the subject have been well summarized by Greenwald (212 791) their explanation must await further investigations

It is clear though from the study of iodine in relation to the prevention of goiter that there must be a definite connection between the two Data which have been summarized by McClendon (211) have shown an apparent reduction of goiter in endemic areas when iodine was added to the salt or water supply It must be pointed out however that iodine acts as a prophylactic in colloid goiter and does very little to reduce the size of the thyroid after the gland has enlarged Inasmuch as this type of goiter has not been produced in animals by experimental diets the pathogenesis of the process is not at all clear The most commonly accepted hypophysis that of Marine

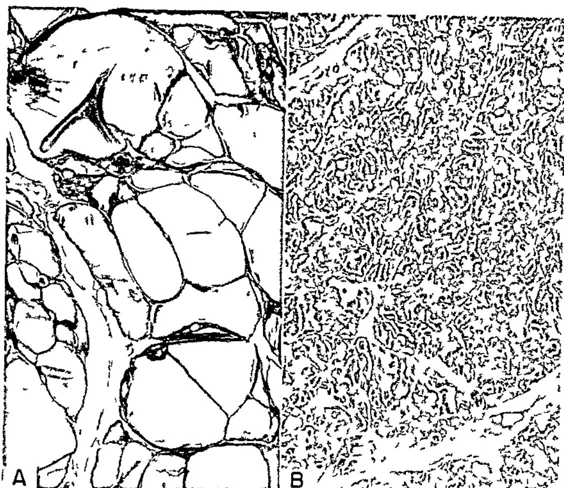


FIGURE 21a Thyroid The colloid and hyperplastic types of goiter *A* A 23 year old white female who had noted swelling of the neck for ten years and a choking sensation for the past two years Examination revealed a large diffuse soft thyroid BMR —5 Note large follicles filled with colloid and lined by flattened epithelial cells *B* A 37 year old colored female who had noted choking sensation swelling of the neck nervousness and bulging of the eyes BMR +60 Treatment with rest and KI reduced BMR to +13 Subtotal thyroidectomy was performed Note great cellularity with infolding of cells into lumen and scanty colloid Both H and E ×50

already noted All must be evaluated before precise conclusions can be drawn Because of such interfering variables little progress has been made in attacking the problem of the relations of iodine to the deposition of colloid in the thyroid gland That absolute or relative iodine deficiency leads to an hyperplastic thyroid seems well established however (201, 202 203 204 205) In the rat various degrees of goiter characterized by epithelial proliferation have been produced but one must always keep in mind the other extraneous factors referred to above Indeed to be hypercritical it is not entirely certain to date whether absolute iodine deficiency goiter (epithelial hyperplasia type) has ever been attained experimentally in normal animals, certainly colloid goiter has not

fluorine deficiency in experimental animals. Some years ago Sharpless and McCollum (215) fed three generations of rats a diet low in fluorine content, no effects were noted. So too, Evans and Phillips (216) fed similar animals a milk diet for 5 generations and found only a decrease in the fluoride content of the skeleton. More recently McClendon (214, 217) utilized the hydroponics technique to prepare fluorine-free foodstuffs and studied the effects of such a diet on several rats. One animal died in 48 days of starvation, because severe caries prevented chewing while a second rat lived a little longer but evidenced severe caries at autopsy. Similar changes have been observed in rats from mothers placed on low-fluorine rations. Histological studies of such teeth have not been as yet reported and these few animals then furnish the only evidence that fluorine-deficient diets have any effect on the tooth structures of the growing animal.

Fluorine Deficiency in Man. The relationship of fluorine to tooth lesions in the human has been based more on the effects of increased amounts of fluoride in the diet rather than on decreased concentrations. In 1931, it was conclusively shown that changes in the enamel of the teeth may be found when there is an increased concentration of fluorine in the drinking water (218). Such lesions, mottled enamel or enamel hypoplasia, consist of pits and depressions in the enamel covering of the teeth. Since 1931 numerous epidemiological studies have shown conclusively that this disease is related to the fluoride content of water and, as the concentrations of fluoride increase, the severity of mottled enamel likewise becomes more severe. The disease is particularly prominent in this country, especially in New Mexico and certain portions of Texas, where the fluoride content of the water may be very high. For instance, in Lubbock, Texas, the water contains 4.4 parts of fluorine per million and, as a result, over 90 percent of the children between the ages of 9 and 11 have mottled enamel (219).

As would be expected from the gross appearance, histological examination of such teeth reveals the ameloblasts to be most affected (220), such cells apparently become inactive and assume a flat shape. In addition, defects in calcification of the dentine appear and this material becomes stratified. There is also damage to the odontoblasts since many have pyknotic nuclei. Though only the teeth of the growing organism are affected, bone may be involved in hyperfluorosis at any age; such osseous changes consist of thickening of the trabeculae in the shaft and the periosteal new bone formation about the cortex (220). In the human, the concentrations of fluoride in the drinking water necessary to produce these changes must be relatively large and a fairly long period of ingestion of such concentrations must prevail before radiological and histological defects can be found in the bones.

An even more important phase of fluorine metabolism in its relation to preventive medicine is the accumulating evidence that certain concentra-

(213), is that iodine deficiency leads to epithelial hyperplasia which after a time is followed by a reversion or involution to normal, the nearest approach to normal being colloid goiter, such a cycle may repeat itself The stages of this cycle have been correlated with the iodine content of the thyroid gland, for instance in human tissues, the normal, hyperplastic gland and colloid goiter contain 2 17, 32 and 1 99 mgm per gram of dried gland, respectively (210) It is unfortunate that there are so few data on the incidence of colloid and hyperplastic goiters in similar localities Such studies based on histological examination of the tissue at operation and autopsy might aid in a better understanding of the pathogenesis of both types According to McClendon's (211) data there is some evidence that the incidence of the two types have some parallelism according to geographical location The evidence is not entirely conclusive, however, since it is based on too few observations A further problem of course, can be cited by the surgical pathologist who seldom encounters foci of colloid follicles in hyperplastic glands or *vice versa* The relation of iodine to morphological changes is very, very obscure

The mode of action of iodine on the hyperplastic gland of Grave's disease is not clear The gland becomes less hyperplastic and the follicles fill up with colloid That this is unrelated to iodine deficiency is obvious however since the iodine effect on the tissues and basal metabolic rate is only a temporary one

Fluorine

Historical The indispensability of flourine for the animal organism has not been as yet completely proved, however, there is suggestive evidence that this element is necessary to protect the integrity of growing teeth The first positive report dealing with a diet deficient in fluorine and its effect on tooth structure of the rat was made by McClendon in 1944 (214)

Biochemical Relationships The biological role of fluoride ions whether in increased or decreased concentrations, is not entirely clear Relatively small amounts of fluoride compounds are tissue poisons doubtless as a result of the inactivation of specific enzyme systems for instance small concentrations of fluoride ions inhibit the calcification of rachitic cartilage in vitro, on the other hand similar concentrations have much less action on bone phosphatase activity (372) The effect of inadequate amounts of fluorine in biochemical reactions has not been studied sufficiently to determine what, if any, the result might be

Pathological Effects Only a few attempts have been made to produce

Part III

THE ESSENTIAL AMINO ACIDS

"A deficiency in a nitrogenous dietary need not necessarily be one of quantity, the form in which nitrogen is supplied may determine its efficiency Thus, in the familiar case of gelatine it is, of course, a qualitative deficiency which makes that substance unable to maintain nitrogenous equilibrium It is generally assumed that this qualitative deficiency is occasioned by the absence from gelatine of certain molecular groups which are present in true protein, but this hypothesis leaves unexplained the advantage possessed by gelatine over fats and carbohydrates as a protein sparer" Willcock and Hopkins 1906 (227)

tions of fluoride compounds in drinking water protect against the development of dental caries. Several years ago because the water supply in Bauxite, Arkansas contained a high content of fluorine the source of the water was changed to one which contains virtually none of this element (221). When, about 10 years after this change, the teeth of the children in Bauxite were examined, the incidence of mottled enamel was found to be as high as expected, more interesting however, was the observation that such children who had been drinking this fluorine free water for 10 years had an extremely low incidence of dental caries (224). In contrast, children living in a near-by town who had used the same source of fluorine-free water all their lives had a high incidence of caries.

These and other observations (758) have stimulated a tremendous amount of interest in the use of fluorine to protect against dental caries, at the present time extensive research programs dealing with this problem are under way in a number of states (222). The most impressive data which are available on caries prevention have been furnished by Knutson (223) of the Public Health Service. In April and May of 1942 a group of children received 7 to 15 topical applications of sodium fluoride solution to the teeth in the left upper and lower quadrants of the mouth. Upon examination 2 years later 41.3 percent less teeth were carious on the treated than in the untreated side of the mouth. *In addition, the number of additional carious lesions in teeth* which were decayed at the beginning of the study was 23.1 percent less in the treated than in the untreated carious teeth. The results of similar studies now being carried on will be awaited with interest.

PROTEINS IN GENERAL

Of the three chief foodstuffs proteins constitute the most important group Besides contributing to the structure of the cell and of intercellular substances (osteoid, dentine, collagen, et cetera) proteins and their constituent amino acids are of tremendous importance in the formation of enzymes, hormones, hemoglobin, plasma proteins, antibodies and many other physiologically active substances

Proteins of course vary widely in their amino acid content, the importance of this in nutrition was brought out by the early observations of Hopkins (227) and of Osborne and Mendel (228) The inadequacy of zein as a sole source of protein because of its lack of tryptophane and lysine is one of the classic observations in the science of nutrition Such experiments initiated modern studies of the dispensable and indispensable amino acids a subject which W C Rose and his collaborators have done much to elucidate

Of the twenty-odd amino acids which have been isolated, Rose has designated ten as *indispensable*, that is those which are needed for normal growth in the rat tryptophane lysine histidine, arginine, phenylalanine, isoleucine leucine, threonine methionine and valine Further studies have shown that these same amino acids are necessary for normal growth of the dog and that all but two arginine and histidine are necessary to promote a positive nitrogen balance in man Arginine is synthesized in moderate quantities by the rat so that it is only indispensable for the growing animal The essential amino acids have been studied in relation to their ability to restore hemoglobin and/or plasma protein formation in dogs rendered anemic and/or hypoproteinemic

It must be stressed that the present list of indispensable amino acids must not be accepted as final More may need to be added, and it is possible that others may be removed from the essential group Evidence now accumulating suggests that perhaps certain amino acids or similar materials are synthesized by the intestinal flora (225 226) For instance when sulfasuxidine is added to the diets of rats which are receiving either the ten essential amino acids casein or a casein digest as sources of nitrogen growth is much poorer in the animals fed the crystalline amino acids Such results may be interpreted to indicate that casein and casein digest supply amino acids other than the ten essential ones and that these additional amino acids are ordinarily synthesized by the intestinal flora

Table V presents a short summary of the effects of deficiencies of the ten essential amino acids in the various mammalian species studied thus far What few details that are known concerning the pathological changes pro-

Part III

THE ESSENTIAL AMINO ACIDS

duced by such deficiencies will be found on the following pages How little has been learned, particularly in comparison with the data which have been accumulated on deficiencies of the elements and vitamins, will be obvious The sections dealing with each amino acid have been arranged arbitrarily in order of the discovery of their individual indispensability

Tryptophane

Historical One of the monuments in the history of nutrition was Willcock and Hopkins' observation that mice fail to grow and even die if the sole source of dietary protein is zein (227) Although growth was prolonged when these investigators added tryptophane to the ration it remained for Osborne and Mendel to furnish the complete story and show that zein plus tryptophane plus lysine promoted normal growth, thus tryptophane and lysine were established as essential nutrients (228)

Biochemical Relationships As is the case of so many essential amino acids the function of tryptophane in the organism is not entirely clear Its importance is obvious since it is a constituent of plasma protein, hemoglobin, thyroglobulin, Nissl substance and many other body proteins The relation of pyridoxine to the metabolism of tryptophane is a most interesting one which is discussed elsewhere (page 183) Even more important is the relationship of this amino acid to the pellagra syndrome in man and to the effects produced in experimental animals by diets containing large quantities of cornmeal It is now clear that since the tryptophane content of cornmeal is low the grain does not furnish the organism enough of this amino acid to form nicotinic acid Pellagra, therefore must be looked upon in part as a deficiency of tryptophane rather than of nicotinic acid (594)

Pathological Effects Experimental tryptophane deficiency leads to the following Failure of growth (mouse rat and swine) alopecia (rat), cataract (rat swine) anemia (rat, dog swine), hypoproteinemia (rat, dog, swine), and changes in the teeth (rat)

Since the experiments of Hopkins (227) and of Osborne and Mendel (228) it has been well established that tryptophane deficiency leads to a disturbance in growth That this amino acid is also necessary for the maintenance of nitrogen equilibrium in the adult organism has been demonstrated in the rat (229) and dog (230)

Loss of hair is a prominent feature of tryptophane deficiency in the rat Alopecia begins on the head and spreads to involve the face and back (231) In the growing animal the hair may be restored by adding tryptophane to the deficient diet No histological studies have been reported thus far

Cataracts have been described in both rats (232 233) and swine (234) In the former species two types acute and chronic have been designated The former starts in the posterior cortex of the lens and spreads to involve

Table V

A Summary of Some of the Effects Produced in Various Species by Deficiencies of the Ten Essential Amino Acids

	RAT			MOUSE	DOG			MAN	
	Growth		Other Effects	Growth	Nitrogen Balance	Plasma Protein Formation	Hemoglobin Formation	Nitrogen Balance	Other Effects
	Young	Adult							
TRYPTOPHANE	+ (228, 231)	+ (229, 280)	Anemia, alopecia, cataract, corneal vasc. (231), hypoproteinemia (227)	+ (269)	+ (230)	+ (236)	+ (235)	+ (238)	
LYSINE	+ (228)	0 (280)	Corneal vasc. (242)	+ (269)	+ (230)		+ (235)	+ (243)	Excretion of organic acids (243)
HISTIDINE	+ (245, 249)	+ (249)	Corneal vasc. (250)	+ (244, 269)	+ (230, 236)		+ (235)	0 (251, 260)	Abnormal urinary metabolites (251)
ARGININE	+ (252)	0 (290)		0 (269)	0 (230)	+ (256)	+ (235)	0 (255)	Decreased spermatogenesis? (255)
PHENYLALANINE	+ (257, 259)	0 (280)		+ (269)	+ (230)		+ (235)	+ (260)	
LEUCINE	+ (261)	0 (280)	Enlargement hypophysis, corneal vasc. (263)	+ (269)	+ (230, 236)		+ (235)	+ (260)	
ISOLEUCINE	+ (264)	+ (280)		+ (269)	+ (230)		+ (235)	+ (260)	
THREONINE	+ (266)	+ (280)		+ (269)	+ (230)	+ (256)		+ (260)	
METHIONINE	+ (270)	+ (280)	Anemia and hypoproteinemia (274)	+ (269)	+ (230, 236)		+ (235)	+ (285, 288)	
VALINE	+ (286)	+ (280)	Irritability and spinning movements (296)	+ (269)	+ (230)	+ (256)	+ (235)	+ (288)	

Tryptophane Deficiency in Man In experimental tryptophane deficiency in the human, studies have been carried out for very short periods Aside from a negative nitrogen balance which indicates that this amino acid is indispensable, no significant changes have been observed (238) The relationships of tryptophane to nicotinic acid synthesis has recently assumed great importance and the possibility that pellagra may be in part the result of tryptophane deficiency, inasmuch as cornmeal contains so little of this amino acid, appears to be a real one More details of this subject will be found on page 183

Lysine

Historical Lysine and tryptophane were the first indispensable amino acids to be recognized From the preceding section it will be recalled that Hopkins showed that the growth of mice can be prolonged when tryptophane is added to zein and that Osborne and Mendel (228) later showed that growth can be much improved when lysine is added to the tryptophane-zein mixture The latter investigators also demonstrated that rats fail to grow on a diet of which the protein is supplied by wheat gliadin and that growth is improved when lysine is added

Biochemical Relationships Little is known of the role of lysine in physiological processes It is unique among the essential amino acids thus far studied in that once it is deaminated, the residue cannot be reaminated to form lysine again (239)

Pathological Effects Lysine is necessary for the growth of rats (240, 241) Although disagreement exists as to whether a deficiency of this amino acid leads to anemia, there is some evidence which indicates slight though definite decrease in red blood cells and hemoglobin concentrations in both growing rats (240) and dogs (235) The only microscopic studies which have been reported are on the bones and eyes of rats Osseous changes are non-specific and resemble those seen in inanition (241) Corneal vascularization which resembles that seen in other deficiencies (242) has been described

Lysine Deficiency in Man Investigations of experimental lysine depletion in the human have demonstrated the development of a negative nitrogen balance (243) After 5 days on the deficient diet all 3 of the subjects so studied complained of nausea dizziness and hypersensitivity to metallic sounds In addition unidentified non ketonic organic acids may be detected in the urine these acids are interpreted to indicate a 'biochemical lesion' of lysine deficiency It is unlikely that lysine deficiency can ever occur in man except under experimental conditions

the perinuclear and anterior cortical zones and usually matures within two or three weeks. The latter, which takes longer to develop and matures more slowly, is confined to the anterior and posterior cortices. Cataracts have been described only in growing rats, not in adult animals. In two of three swine studied by Wintrobe et al. (234) opacities were noted in the equitorial portion of the lens, in one animal these spread to the anterior and posterior portions of the lens. Corneal vascularization is said to occur in tryptophane deficient rats (233), but has not been observed in swine. A slight anemia has been reported in rats maintained on a diet whose protein content was furnished by acid hydrolzed casein, the blood returned to normal when tryptophane was administered (229). A much more marked reduction in red blood cells and hemoglobin has been observed in swine fed an acid hydrolysate of casein (234). This anemia is normocytic or slightly microcytic and normochromic. The reduction in red blood cells is accompanied by normal serum iron levels, no hemosiderosis is found in the tissues. The bone marrow is not particularly hyperplastic. In dogs rendered anemic by bleeding accelerated hemoglobin formation takes place when tryptophane is administered (235).

A reduction in plasma proteins has been observed in rats (229) and swine (234) made deficient in tryptophane. In the latter species the protein concentrations may be reduced, for instance, in one animal from the normal of over 6 gm percent to as low as 2.8 gm percent after 117 days on the deficient diet. Tryptophane leads to prompt regeneration of plasma protein in the hypoproteinemic plasmapheresed dog (236).

Absence of development of the yellow pigmentation of the incisor teeth has been observed in the rat (231). This, as might be expected, is only observed in growing animals and its etiology is not clear.

In three swine deficient in tryptophane, necroses and atrophy of the skeletal muscle fibers have been observed (234), changes which are similar to those noted in vitamin E deficiency.

Albanese et al. (337) have claimed that "tryptophane is a most important dietary essential for normal gestation in the rat." Until more evidence is presented such a claim is not justified. The differences observed are probably the result of inanition since the deficient rats lost, on the average, 34 gm., while the controls gained on the average 31 gm. In addition the food consumption of the latter group was greater than that of the former. It seems logical to assume that reproduction cannot take place when any of the ten essential amino acids are absent since by definition all are necessary for normal growth.

In summary, experimental tryptophane deficiency leads to failure of growth, alopecia, cataract, anemia, hypoproteinemia and loss of the pigment in the rat's incisor.

indican test, has not been further identified It is unlikely that histidine deficiency can ever occur in the human except under experimental conditions

ARGININE

Historical The indispensability of arginine for the normal growth of young rats was first shown by Rose and his group (252) This amino acid is not necessary in adult rats, since sufficient amounts can be synthesized to maintain the organism in nitrogen balance (280)

Biochemical Relationships Arginine has been shown to lead to deposition of a small amount of liver glycogen and to decrease ketosis in fasting rats fed sodium butyrate (253) Arginine is probably one of the precursors of creatine (254) and, of course, acts as a catalyst in the synthesis of urea

Pathological Effects As noted above arginine is an indispensable nutrient for the growing rat, but is not necessary for the adult rat (280), mouse (269) or dog (230), since these organisms can synthesize sufficient quantities of the amino acid Arginine, therefore, has a unique place between the indispensable and dispensable amino acids There is some evidence that arginine is necessary for plasma protein formation in the protein depleted dog (256) and for hemoglobin formation in anemic dogs (235)

Arginine Deficiency in Man Studies in man have indicated that although arginine deficiency does not lead to negative nitrogen balance, the amino acid may have an important relation to spermatogenesis Holt et al (255) have reported a ten-fold reduction in the number of spermatozoa of the seminal plasma of 3 individuals after 9 days on an arginine-deficient diet Following the inclusion of arginine in the diet there was a return to normal after several weeks It is unfortunate that such observations are marred by the absence of any control studies before the arginine-deficient regimen was instituted Further observations in man and in experimental animals are clearly indicated

PHENYLALANINE

Historical One of the many important reports from Rose's laboratory showed that phenylalanine is an indispensable amino acid and that tyrosin is not (257) this cleared up a subject which until 1934 had been much confused

Biochemical Relationships The normal metabolism of phenylalanine probably calls for its conversion into tyrosin and further break-down by opening the benzine ring (258) The importance of ascorbic acid for phenylalanine metabolism has been shown in both guinea pigs and premature babies and is discussed elsewhere (page 131) Phenylalanine and tyrosin are structurally very closely related to epinephrine ephedrine thyroxin and

HISTIDINE

Historical Histidine was added to the group of indispensable amino acids in *1917* when the investigations of Geiling (244) clearly showed that adult mice will fail to maintain their weight when this amino acid is omitted from their diets The indispensability of histidine for the rat was soon confirmed by Cox and Rose (245)

Biochemical Relationships Histidine is apparently a precursor of carbohydrate since liver glycogen deposition follows its ingestion and the ketonuria produced by a high fat diet is decreased when histidine is fed (246)

The relation of histidine to histamine is not clear Certain tissues contain an enzyme which will form histamine from histidine, for instance kidney tissues contain an enzyme histidine decarboxylase which affects this transformation (247), so it is quite likely that toxic effects which are noted when histidine is administered, may result from its conversion to histamine This may explain the observation that in rabbits to which this amino acid is administered anorexia tachycardia respiratory difficulty, and paralysis of the hind extremities may be observed and may be followed by death Microscopic examination of such animals reveals pulmonary edema and constriction of the bronchial musculature (248)

Pathological Effects Histidine is necessary for growth of the rat (245 249, 250) Such animals acutely deficient in histidine develop a moderate anemia, hemoglobin values fall from the normal of 14 gm to about 10 gm percent Histological examinations of the tissues have failed to reveal any specific changes other than those ascribed to inanition except in the eye Here the corneal epithelium is thinned and the superficial portion is keratinized in addition there is leukocytic infiltration and blood vessels grow into the substantia propria (250)

Histidine is necessary for the weight maintenance of adult rats (249) and deficiency in this amino acid leads to a negative nitrogen balance in dogs (250) Histidine is probably necessary for plasma protein production in the plasmapheresed dog and leads to hemoglobin formation in dogs made anemic by bleeding However the relation of histidine to hemoglobin formation in this species requires further study

In summary, the only morphological changes resulting from histidine deficiency is corneal vascularization Anemia and hypoproteinemia may develop Further studies are greatly needed

Histidine Deficiency in Man In experimental histidine deficiency over short periods, histidine does not lead to a negative nitrogen balance (251, 260) There is loss of weight, however and an abnormal metabolite appears in the urine (251) This substance which gives a green color with the Sharlit

velops when leucine is omitted from the diet (260) It is unlikely that a deficiency in this amino acid ever occurs in the human except under experimental conditions

Isoleucine

Historical The indispensability of isoleucine for the growth of rats was demonstrated by Womack and Rose in 1936 (261)

Biochemical Relationships Little is known of the rôle of isoleucine in nutrition It is of interest that human plasma proteins (264) as well as hemoglobin (265) contain insufficient amounts of isoleucine to promote normal growth in the rat

Pathological Effects Womack and Rose (260) showed that isoleucine is necessary for growth in the rat In the dog isoleucine is necessary for hemoglobin (235), plasma protein formation (236) and the maintenance of nitrogen balance (230) No histological studies have been reported on any species deficient in isoleucine

Isoleucine Deficiency in Man The indispensability of this amino acid for the nutrition of humans has also been established since in its absence negative nitrogen balance develops (260) As in the case of the majority of the amino acids it is very unlikely that a deficiency of isoleucine can ever exist except under experimental conditions

Threonine

Historical While studying the problem of essential and non-essential amino acids Rose and his co-workers discovered a new indispensable amino acid (266) After proving its analogous chemical structure to d-(-)-threose, the new substance was named threonine (267)

Biochemical Relationships Little is known of the role of threonine in physiological processes except that it may be converted into carbohydrate by the rat (268)

Pathological Effects Virtually no attention has been given to the study of pathological effects produced by threonine deficiency Growth disturbance in the rat (266) and its influence on serum protein production in the dog (256) have been observed when such animals are placed on diets deficient in threonine Mice exhibit a rapid loss of weight and develop a queer puffy appearance with marked abdominal distention (269) At autopsy there is pronounced edema and ascites it is likely that these findings may result from hypoproteinemia

Threonine Deficiency in Man In man a negative nitrogen balance has been observed when threonine is withheld from the diet (260) of experimental subjects It is extremely unlikely that threonine deficiency could ever occur other than experimentally in man

melanin, although changes in the metabolism of these substances have not been reported in animals deficient in this amino acid

Pathological Effects In rats whose diets are deficient in phenylalanine there is a cessation of growth (257, 259) Pathological studies have been reported in a few deficient rats and their paired fed controls placed on a phenylalanine-deficient diet for 28 days (259), in these there is an average reduction in hemoglobin from 14 7 gm to 9 9 gm, and a slight fall in plasma protein concentration Histological examinations of the tissues show no changes other than those which can be ascribed to inanition disturbance in endochondral bone formation, thymic atrophy, atrophy and decreased fat content of the adrenals, and atrophy of the testicular tubules

In dogs this amino acid has been shown to be necessary for plasma protein production (336) and for the maintenance of nitrogen balance (230)

Phenylalanine Deficiency in Man In man a negative nitrogen balance has been observed when dietary phenylalanine is restricted (260) It is unlikely that a deficiency of this amino acid can ever occur except under experimental conditions

Leucine

Historical Leucine was shown to be an indispensable nutrient to the growth of rats by Womack and Rose in 1936 (261)

Biochemical Relationships The role of leucine in nutrition is obscure except that it has been shown to furnish carbon atoms for steroid formation (242)

Pathological Effects Leucine is necessary for the growth of rats (261, 263) In animals whose nitrogen requirements are supplied by the essential crystalline amino acids other than leucine specific effects on the ocular tissues and hypophysis have been reported by Maun et al (263) In the cornea the epithelium becomes thinner and subsequently keratinized The basal layers evidence an increased mitotic activity There is an accompanying vascularization of the substantia propria—apparently most marked just beneath the epithelium In addition, the iris and ciliary body exhibit leukocytic infiltration No mention has been made of the condition of the various ocular glands The hypophysis of the leucine deficient animal is said to be, on the average twice as large as the gland of the controls No cellular alterations appear to occur and it is further stated that the distribution of the various types of cells is normal Whether the increased weight is due to hypertrophy or hyperplasia has yet to be elucidated

In dogs leucine appears to be necessary for plasma protein and hemoglobin formation (236 256), in the rat on the other hand no evidence exists that leucine deficiency affects these two functions

Leucine Deficiency in Man In man a negative nitrogen balance de-



FIGURE 21b Cystine Deficiency *A* Liver from rat on a low protein (5 per cent) high fat (30 per cent) choline supplemented diet There is no fat accumulation in the intact hepatic cells Most however show widespread fresh necrosis without hemorrhage *B* Similar liver with exception that there is extensive hemorrhage in the necrotic areas

If groups of rats are placed on diets whose protein content is supplied by mixtures of crystalline amino acids containing varying amounts of methionine and cystine the following differences are noted (274) When both methionine and cystine are absent, the animals lose weight and develop an uncharacterized anemia hypoproteinemia and extensive hemorrhagic necrosis

Methionine

Historical Until 1937 both of the sulfur-containing amino acids methionine and cystine, were considered to be indispensable components of the diet At that time however, Rose and his co-workers (270) demonstrated that cystine is a dispensable nutrient, for when adequate amounts of methionine are added to a cystine-free amino acid mixture, growth of rats fed this ration is normal That methionine is converted into cystine in the normal rat, further clarifies their relationship

Biochemical Relationships The metabolism of methionine is closely related to that of cystine, and in turn to choline, creatine and similar substances including a host of sulfur-containing compounds such as those enumerated on page 41 Methionine is indispensable except under stringent laboratory conditions i e when homocystine and choline are fed to rats on a methionine-free diet, the latter amino acid will be formed (271)

Methionine donates methyl groups to ethanolamine resulting in the formation of choline (272) (page 193) The stages in the transformation methionine →cystine are thought to be as follows methionine →homocysteine methyl groups, homocysteine serine →cystathione →cysteine →cystine The sulfur of the cystine which results is derived from methionine, the carbon chain from serine

Pathological Effects Since the metabolisms of methionine and choline are so closely related it is extremely difficult to separate the pathological manifestations of a deficiency of one from the other So too, since methionine is a precursor of cystine it is difficult to separate the effects of deficiency of either of these two amino acids When methionine is absent from the diet cystine obviously will be, too unless otherwise supplied

From data which are already accumulated it appears that the effects of cystine and choline deficiency may be different (page 221) Investigations of cystine deficiency begin with the experiments of Weichselbaum (293) who noted jaundice before death and hemorrhages in the liver of rats postmortem The administration of cystine to such animals in the basal diet prevented the appearance of such lesions in addition moribund rats could be saved by the administration of cystine but not methionine The cystine methionine, choline deficiency question then became somewhat complex but Daft and his co-workers (294) in 1942 were able to separate the effects of cystine deficiency hemorrhagic necrosis of the liver from choline deprivation which leads to extreme fatty infiltration and cirrhosis

Experimental procedures employing crystalline amino acid mixtures supplemented with methionine and/or cystine in the presence of adequate choline have been reported (274) and confirm the previous work the data are open to some criticism, however, as too few animals have been employed

such dogs since if within 3 to 4 hours following the anesthesia this amino acid is injected intravenously, the animals recover It is unlikely that such protective effects can be observed in normal dogs (284)

In summary, methionine itself appears to be necessary for hemoglobin and plasma protein production This amino acid is necessary for the formation of cystine unless the latter is supplied in the diet Lack of cystine appears to lead to liver necrosis and to changes in the hair

Methionine Deficiency in Man When experimental subjects are placed for short periods on methionine-deficient diets a negative nitrogen balance develops (285, 288) It is difficult to conceive of uncomplicated methionine deficiency occurring other than experimentally in man

VALINE

Historical The indispensability of valine in the diet of the rat was demonstrated in 1939 by Rose and Eppstein (286)

Biochemical Relationships Little is known of the role of valine in nutrition save that in the phlorhizinized dog valine has been shown to contribute three of its five carbon atoms to the formation of glucose (287)

Pathological Effects When rats are placed on a valine deficient diet they virtually stop eating and rapidly lose weight In the terminal stages of the deficiency they exhibit unique signs which Rose and Eppstein (286) have described as follows 'The rats become extremely sensitive to touch and display a severe lack of coordination in movement They walk with a staggering gait As the animal attempts to walk the left foreleg is raised inordinately and the head is retracted Frequently the subjects show a rotary motion resembling that of a dog chasing its tail This may be either clock or counter-clockwise and may continue until the animal falls to the floor of the cage from sheer exhaustion As would be anticipated the symptoms are rapidly cured by the administration of valine without any other therapeutic measure' No histological studies in such animals have been reported and of course are greatly to be desired

Valine is necessary for plasma protein and hemoglobin formation in the dog (255 256)

Valine Deficiency in Man When the amino acid is removed from an otherwise adequate human diet a negative nitrogen balance develops, this is interpreted to indicate that valine is indispensable for this species as well (288) It is hardly conceivable that valine deficiency could occur in man unless experimentally induced

of the liver When inadequate amounts of methionine but no cystine are added to the diet, the animals live a little longer but succumb with liver necrosis although no anemia or hypoproteinemia develop When methionine is restricted but there is adequate dietary cystine, no liver necrosis is observed although there is a disturbance in hemoglobin and plasma protein formation Apparently then methionine deficiency interferes with hemoglobin and plasma protein function while lack of cystine leads to liver necrosis which develops in the absence of fatty infiltration (page 193) as choline is present in the diet

Since methionine is a precursor of cystine what little is known of the role of the latter amino acid in the organism should be mentioned Some time ago cystine was shown to be a constituent of hair, various specimens of human hair which have been analyzed contain on the average almost 20 percent of this amino acid (275) That rats placed on a cystine-deficient diet will show an inhibition of hair growth is therefore not an unexpected finding (276) The hair of such animals is also abnormal in that the medullary cells are broader and more loosely packed, the cortical portion, which incidently contains sulfur is narrower than control hair It is unfortunate that histological studies of the skin of such animals have not been made Other experiments employing diets deficient in sulfur-containing amino acids indicate a slightly increased production of hair when methionine is added into the diet (277) Another most interesting aspect of this problem was studied in a strain of hereditary hypotrichotic rats When cysteine is administered to these animals growth of the hairy coat is stimulated It is concluded that cysteine manifests this action because of the sulfhydryl group it contains (278) These observations have not been confirmed (279)

Some incidental observations of methionine deficiency in other species might be mentioned This amino acid is necessary for the maintenance of nitrogen metabolism in the adult rat (280) and the dog (230) In addition it is apparently necessary for growth of the mouse (269) and for hemoglobin formation in anemic dogs (281)

The relationship of methionine to the maintenance of the integrity of the liver of protein-depleted dogs in the presence of poisons is an interesting one Any results of course may be due to a choline or cystine effect In dogs which are placed on a low protein intake and whose protein stores are reduced by plasmapheresis there is a reduction in tolerance to the intravenous administration of mapharsen as measured by the appearance of jaundice (282) The administration of methionine the day before the injection of the arsenical raises the tolerance of the animal in that larger doses of the drug are required to produce icterus Then too if similar protein-depleted dogs are maintained under chloroform anesthesia for 30 minutes they die of necrosis of the liver (283) Methionine has a dramatic effect on

PART IV

THE FAT AND WATER-SOLUBLE VITAMINS

'All these diseases, with the exception of pellagra, can be prevented and cured by the addition of certain preventive substances, the deficient substances, which are of the nature of organic bases, we will call 'vitamines', and we will speak of a beriberi or scurvy vitamine, which means a substance preventing the special disease" Funk 1912 (1)

INTRODUCTION

A vitamin is usually defined as an organic substance, soluble in fat or water which is ordinarily needed only in minute quantities to maintain the metabolic integrity of certain cells and tissues. The term vitamer has been introduced to refer to substances having more or less similar biological activity but differing structurally, as for instance pyridoxine, pyridoxal, and pyridoxamine.

During the beginning of the second decade of this century, McCollum and Davis (300) and Osborne and Mendel (301) demonstrated a fat-soluble material that promoted growth in animals which had been placed on purified diets. This material was named fat-soluble vitamin A. In 1922, McCollum and his co-workers showed that vitamin A could be destroyed if oxygen was bubbled through cod liver oil and that a second fat-soluble material remained in cod-liver oil so treated; this substance or vitamin D proved advantageous in curing and preventing the manifestations of rickets. In the same year Evans and his group (390) showed that reproductive activity in rats was interfered with when animals were placed on synthetic diets containing certain fats; the addition of wheat-germ oil to such rations restored reproductive activity to normal. The active substance in wheat-germ oil was named vitamin E (the fifth vitamin to be isolated). About ten years later the fourth member of the fat-soluble group, vitamin K, was discovered (436).

The characteristic of fat solubility which these four substances display is of some importance since any interference with the absorption of lipids from the intestinal tract will hinder the absorption of these vitamins as well. For instance, if bile and/or pancreatic juice are absent or present in inadequate quantities a conditioned deficiency of all four of the fat-soluble vitamins may occur. In man signs of deficiency in vitamin A, D and K have repeatedly been seen under such circumstances. Adequate evidence for the occurrence of vitamin E deficiency in the human is lacking. Although the structural changes associated with deficiency of the fat-soluble vitamins have been described and studied rather fully, the underlying mechanisms of the biochemical defects are less clearly understood, especially in comparison with what is known of the function of the water-soluble group.

The story of the development of our knowledge of the group of water-soluble vitamins as a whole is far more complex than that of the fat-soluble series. What follows is a very brief resume of long and heart-rending investigations. By 1915 McCollum had separated 2 growth provoking substances, fat-soluble vitamin A, and water-soluble vitamin B which occurred in yeast (759). Ten years later the water-soluble substance in yeast was

PART IV

THE FAT AND WATER-SOLUBLE VITAMINS

Vitamin A

Historical In 1913 two groups of investigators, McCollum and Davis (300) and Osborne and Mendel (301) reported that certain fats contain an essential nutrient for rats, without this substance animals fail to grow in normal fashion Several years later, Steenbock (302) suggested that the active principle or vitamin A as the material had then been designated, was carotene a yellow pigment derived from plant and animal tissues During the next ten years the exact identity of vitamin A remained unsettled and in much confusion, in 1930 carotene was shown to be pro-vitamin A (303) Karrer (304 305) then worked out the chemical constitution of both carotene and vitamin A and completed the story in 1936 when a pure material was synthesized (306)

Biochemical Relationships There are a number of closely related substances (carotenes) which behave as pro-vitamin A, of these, the most potent is beta-carotene The carotenes and vitamin A are absorbed from the intestine and the majority of each is stored in the liver Here the carotene portion is broken down into vitamin A by an enzyme carotenase (307) As in the case of the other fat-soluble vitamins bile and/or pancreatic juice facilitate absorption from the intestines, vitamin A deficiency may develop when these secretions are diminished or absent (308) Although the major portion of the organism's store of vitamin A is found in the liver other tissues also contain varying amounts as has been demonstrated by chemical and histological studies The following values indicate the tissue distributions of vitamin A (in British Units per gram) as determined by chemical procedures on rats (309) liver, 40 000, kidney 50, lung 450 adrenal, 2500, heart 1 spleen 2 pancreas 25, thymus 12 brain 0 3, muscle 0 5, blood 2

The fluorescent properties of vitamin A have been utilized to study the histological distribution of this nutrient When formalin-fixed sections of tissue are viewed under a source of ultraviolet light of wave length 328 millimicrons a fading green fluorescence is observed which is interpreted to be due to the presence of vitamin A This histochemical test is apparently not as sensitive as ordinary chemical determinations since the technique fails to reveal the vitamin in tissues where chemical tests indicate that it is present

Little is known of the function of vitamin A in biological systems, except for the role it plays in visual processes Based on observations that when dietary vitamin A is restricted the ability to see under subdued illumination is diminished a theory has been developed for the physiological activity of vitamin A in rod vision (311)

Rhodopsin a photosensitive carotenoid protein pigment is found in the

shown to be dual in nature, since upon autoclaving its B vitamin content was destroyed (761) The heat stable portion was soon broken down into a fairly large group of materials most of which were identified during the 1930s and early 1940s riboflavin in 1935, nicotinic acid in 1937, pyridoxine in 1939 pantothenic acid in 1940, and biotin in 1942 In addition, the dietary importance of choline had been demonstrated in 1932, and inositol and para aminobenzoic acid were added to the list in 1940 and 1941, respectively The most recent member of the B group is *L casei* factor or folic acid which was synthesized in 1945

Ascorbic acid of course stands apart biologically from the above vitamins Scurvy was the first dietary deficiency disease to be recognized with any certainty, and vitamin C was designated as the active principle, ascorbic acid was not identified until 1932

A number of other water-soluble substances *have been described* and designated as vitamins However evidence of their true identity has not been presented in sufficient detail to warrant a discussion of them in this volume

The functions of the water-soluble vitamins are more clearly understood than are those of the *fat-soluble* group A number *have now been shown* to act as parts of coenzymes in biological processes These include thiamine (cocarboxylase) nicotinic acid (phosphopyridine nucleotides), riboflavin (xanthine oxidase d amino oxidase) and pyridoxine (codecarboxylase)

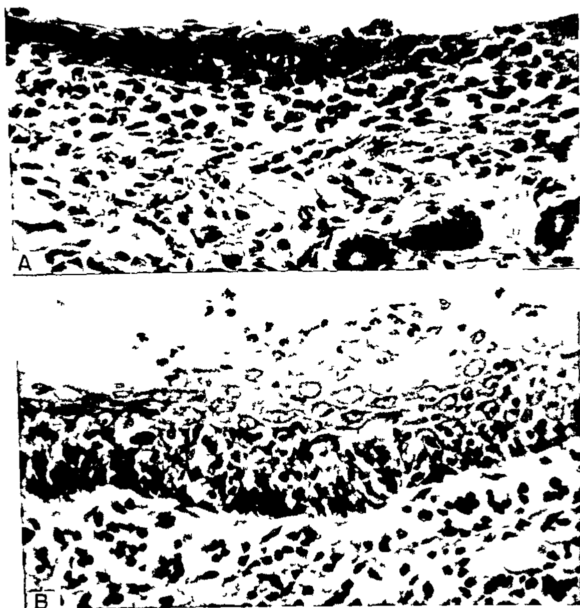


FIGURE 22 Trachea *A* and *B* show different degrees of epithelial metaplasia in the vitamin A deficient rat The normal ciliated and columnar lining has been replaced by a multilayered one which is infiltrated by leukocytes

decrease in size is at the expense of the cytoplasm, the nucleus does not appear to change its dimensions Such foci then spread to embrace large areas of the epithelial lining where small syncytial-like masses of cells appear, derived from the atrophic elements These cell masses rapidly proliferate and undermine the overlying atrophic columnar cells Growth activity of this metaplastic epithelium seems to be augmented, and the cell groups rapidly develop into a keratinized type of epithelium which spreads

rods of a number of mammalian species When light acts upon rhodopsin (visual purple) photic and thermal reactions occur and it is bleached to a compound composed of protein and a carotenoid substance named *retinene*. The latter further breaks down into vitamin A The visual cycle is completed by vitamin A and protein uniting to form rhodopsin Vitamin A appears to be destroyed in this reaction hence a continual supply is necessary In the presence of inadequate vitamin A, rod vision is diminished or absent In other words the eye becomes "night blind," since the rods are responsible for vision under subdued illumination

Pathological Effects As a result of the studies of Wolbach and Howe and others a deficiency of vitamin A has been shown to affect the integrity of certain epithelial tissues as well as bone and teeth

Epithelium Microscopic changes in epithelial structures have been described in the rat (312), guinea pig (313), fox (314), mouse (315) and monkey (316)

As interpreted by Wolbach (317), specific changes are found in those epithelia whose cells have a secreting (chemical) function in addition to the role of a covering layer and whose functioning cells are without power to divide" Epithelial cells comprising the following organ systems have been reported to be affected

a Digestive system parotid submaxillary sublingual and all accessory glands of the tongue buccal cavity and pharynx ducts of pancreas

b Respiratory tract nares, sinuses, Jacobsen's organ, larynx trachea and bronchi

c Genito-urinary system renal pelvis ureter bladder, urethra epididymis, prostate seminal vesicles, coagulating glands uterus, oviducts glands of vulva and vagina

d Special senses eyes including cornea and accessory glands (harderian intra- and extraorbital and tarsal) and retina

e Endocrine system thymus hypophysis (pressure)

The reason for the involvement of and the degree of damage to various epithelial tissues is not clear No relationship has been found as yet between the chemical and/or microscopic distribution of vitamin A and the occurrence or non occurrence of tissue changes An explanation based on embryological grounds is untenable, nor does the order in which various organs are affected by the characteristic keratinizing metaplasia give any clue Wolbach (317) has epitomized the pathogenesis of the epithelial lesions as "atrophy of the epithelium concerned, reparative proliferation of basal cells and growth and differentiation of the new products into a stratified keratinizing epithelium"

If one takes the trachea as an example microscopic examination reveals focal areas of atrophy of the columnar cells to be the initial change The

which has led to extensive studies of the relationship of vitamin A to normal visual processes Night blindness has been observed physiologically in animals (321) and of course in man It is therefore not surprising that when severe degrees of vitamin A deficiency are produced in rats anatomical changes may be elicited in the retina (322) Depending on the degree of the deficiency, degeneration of the retina first manifests itself in the visual neuro-epithelial cells, the rods and then progresses in order through the outer nuclear layer the outer molecular layer and the inner nuclear layer The

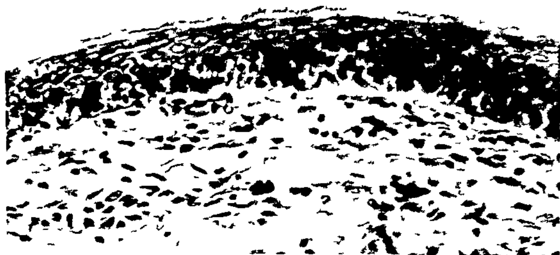


FIGURE 25 Cornea This vitamin A deficient rat shows extensive metaplasia of the corneal epithelium There is also vascularization of the underlying substantia propria and leukocytic infiltration

ganglion cells themselves, are unaffected even in severely deficient animals The outer segments of the rods which have degenerated, are capable of regeneration following therapy, if however more extensive changes have occurred treatment is ineffectual It will be recalled that the rat for the most part a nocturnal animal has very few cones too few for them to appear often in sections so that the reaction of these structures to vitamin A deficiency has not been described

Skin Despite changes in the epithelium at other sites relatively few cutaneous alterations have been ascribed to a lack of vitamin A in experimental animals In the human however Frazier and Hu (323) describe a rough dry skin which microscopically shows hyperkeratosis and hyperkeratotic plugs in the hair follicles For over a decade such changes have been interpreted as pathognomonic of vitamin A deficiency The specificity of these lesions has recently been questioned on both theoretical as well as experimental grounds

so that as the deficiency progresses, the entire trachea may come to be lined by metaplastic epithelium, whose keratinization results in the accumulation of a detritus of keratinized material in the lumen of the trachea. The increased growth activity of epithelium which has been described by Wolbach is questioned by Friedenwald and his associates (318). Quantitative studies of mitotic activity of healing wounds in the cornea of vitamin A deficient rats reveal that the overall mitotic rate for each thousand basal cells is reduced by thirty percent from the normal and the speed of the mitotic cycle is likewise inhibited. Such observations furnish the first quantitative evidence on this fundamental point.

When vitamin A is administered, repair is extremely rapid and diffuse in contrast to the focal development of the initial changes when the animal is placed on a deficient regimen (319). All cells composing the basal layers which are apparently analogous to the stratum germinativum of the skin have the power to proliferate. Such cells differentiate into their former columnar forms and the overlying strata which have apparently irreversibly differentiated, are sloughed off and removed by autolytic phenomena. The tracheal epithelium thus regains its normal morphological appearance.

Some mention must be made of the relationship of the changes in the epithelium of certain specific tissues to the subsequent course of events. In the respiratory tract, for instance, the normal ciliated epithelium lining the trachea and bronchi is replaced by keratinizing cells. Consequently the continuous streaming of surface material towards the pharynx is abolished. Since a protective mechanism of the host has been eliminated pulmonary infections are consequently a common accompaniment of vitamin A deficiency. In the urinary tract renal and cystal calculi have been frequently observed in animals chronically deficient in vitamin A. Such stones result from the inspissation of keratinized material derived from the lining of the renal pelves and bladder.

Ocular Apparatus. Lesions of the eye are of particular importance, especially from the diagnostic standpoint. Xerosis and keratomalacia were the first manifestations of vitamin A deficiency to be described in man. The corneal epithelium shows the characteristic metaplasia seen elsewhere (551). In addition, there is vascularization of the substantia propria, but this is interpreted as a secondary phenomena which results from the keratinization of the epithelium in association with infection (557). It is interesting to speculate as to how much such changes are due to vitamin A deficiency and how much they may be secondary to a diminution of the secretion of the ocular glands because of obstruction of the latter's ducts. Keratitis of course occurs when the secretion of tears is abnormal, as for instance in Sjogren's syndrome in the human (320).

Another of the early manifestations of vitamin A deficiency is nyctalopia

reveals an atrophic single layered, epithelial covering Following the withdrawal of vitamin A, there is some return to normal thickness More important, however, extensive keratinization occurs in the epithelium, especially that of the hair follicles Such lesions have a superficial resemblence to the changes described by Frazier and Hu (323) in the human

Reproductive System Extensive investigations have been reported on the effect of vitamin A deficiency on the male germinal epithelium and on reproduction in the female rat When male rats are placed on a diet deficient in vitamin A, there is atrophy of the germinal epithelium a change which occurs fairly rapidly, according to Mason (325) more rapidly than similar morphologic alterations which result from inanition The difference is apparently one of degree since in both inanition and vitamin A deficiency some of the basal cells persist so that when food intake or vitamin A are restored there follows a rapid return to normal unlike the irreversible changes which occur in the vitamin E-deficient testis (page 122)

In the female there is an alteration in the vaginal smear so that rats markedly deficient in vitamin A show a continuous cornification of the cells (326) As a result the estrous cycle in such animals including the human cannot be interpreted In moderate deficiency states in experimental animals one encounters periods of partial cornification which can be interpreted as meta and diestrous Periods of complete cornification doubtless coincide with proestrous and estrous The vitamin A-deficient female is capable of normal ovulation, implantation and endocrine activity However depending on the severity of the deficiency reproductive function is interfered with Death of the fetus occurs in utero, such fetuses may be either resorbed or expelled Gestation may be prolonged and a few young may be born alive to die shortly after Mason interprets the primary change as occurring not in the embryo proper as in vitamin E deficiency but rather as a result of alteration in the lining of the reproductive tract For at the junction of the fetal and maternal tissues there are localized areas of infection and necrosis in which bacteria have been stained Such areas of destruction of tissue interfere with the nutrition of the embryo It may be, too that there is a bacteremia of the embryo Such a possibility has not been explored It would be most interesting to culture the uterine cavities to determine the prevailing bacterial flora

Females on vitamin A-deficient diets may be bred and from some litters have been removed by Caesarean section All of such young have abnormal development of the eyes lack of differentiation of the lids and cornea and disorganization of the retina (337)

Bone and Nervous Tissues In their early studies of vitamin A deficiency Wolbach and Howe (312) noted impairment of epiphyseal bone formation and interpreted this to be a manifestation of the general inanition which their



Figure 24 Tongue of vitamin A deficient rat Note large cystic structure filled with keratinized debris The normal lining epithelium is keratinized and the orifice of the duct has become obstructed Smaller cysts are seen on either side H and E

Sullivan and Evans (324) call attention to the present concept of the pathogenesis of the lesions of vitamin A deficiency atrophy followed by metaplastic epithelial hyperkeratinization They find it difficult to imagine how such changes can occur in the skin since this structure is, of course, already keratinized It thus appears that metaplastic keratinization cannot occur unless the epithelium is atrophic A vitamin A-deficient ration which was essential in all other respects particularly the B group and the essential fatty acids was therefore concocted Grossly and microscopically no dermal lesions appear when rats are placed on this diet When however, the ration is made deficient in other factors especially the heat-stable B components changes do develop For example, in such an experiment rats are given adequate vitamin A, but inadequate amounts of the B group until marked deficiencies of the latter vitamins are present Vitamin A is then withdrawn and the animals are continued on maintenance levels of the B complex Microscopic examination of the skin before vitamin A therapy is stopped



FIGURE 25 Skeleton Vitamin A deficient rat In *A* the nerve roots (H) show deformities due to herniation through the vertebral foramina In *B* which is a cross section of a vertebra the herniation is better seen (Courtesy of Dr S B Wolbach and the *Archives of Pathology*)

bach feels that appositional bone formation continues in the shaft and elsewhere until inanition supervenes (771)

That vitamin A has a potent effect on bone growth has been even more conclusively demonstrated by Wolbach's observations in various species in hypervitaminosis A (317 771) When large amounts of the vitamin are ad-

animals exhibited. At about the same time neurological signs together with lesions in the nervous tissues began to assume a prominent place in the syndrome of experimental vitamin A deficiency. Mellanby (327) as well as others had studied and reported degeneration of the cranial and peripheral nerves together with lesions in the gray and white matter of the brain and spinal cord. It was postulated, therefore, that vitamin A had a specific effect on nervous tissues, despite the fact that there was no agreement whatsoever as to the pathogenesis or the distribution pattern of the lesions. In 1941 Wolbach and Bessey (328) clarified the matter and stated that they were forced to "the paradoxic conviction that the genesis of the nerve lesions of vitamin A deficiency requires an essentially normal rate of growth of a normal nervous system and that mechanical injury, the result of a disproportion between the central nervous system and its bony enclosure, is the explanation." This interpretation is based on several important observations. In the first place, lesions can only be produced in young, actively growing animals. When rats are placed on a vitamin A-deficient regimen after a certain critical age, neurological manifestations fail to appear, but when the dietary vitamin A content of weanling animals is restricted, evidence of involvement of the nervous tissue appears with regularity during the eighth week. Then too, the pattern of the lesions has no rhyme or reason either in a single animal or when several animals are compared one with another. The reason for this was demonstrated by careful dissection of the nervous tissues within their bony coverings. The cerebellum may be found herniated into the foramen magnum. There may be multiple herniations of the cerebrum and cerebellum into the dural venous sinuses at the site of the arachnoidal villi. There is usually an overcrowding of the spinal canal by its contents so that the spinal cord is distorted and the nerve roots herniate into the intervertebral formina and erode the vertebral bodies. That these phenomenona are a result of vitamin A deficiency alone has been conclusively proved since disturbances in growth produced by inanition or other specific nutritional deficiencies effect the rate of growth of skeletal and nervous tissues alike. Furthermore, that the bone is at fault rather than that there is an overgrowth of nervous tissue can be shown since growth of the latter is normal; in addition the regenerative capacity of the axon is unimpaired.

It is clear that Wolbach and Bessey's (328) observations prove that vitamin A has a specific effect on endochondral bone formation. As yet Wolbach has been unable to detect any specific or characteristic effects on this phase of osteogenesis. The changes appear to be non-specific and resemble those seen in any bone which has stopped growing as a result of lack of calories or nutrients other than vitamin D or ascorbic acid. However, Wol-



FIGURE 76 Lower incisor tooth for a vitamin A deficient rat There is a wide band of dentin under the enamel (empty space) A characteristic proliferation of odontoblasts is also seen (Courtesy of Dr Paul E Boyle)

on the lingual and lateral margins Odontogenic epithelium is also responsible for the organization of mesenchymal pulp cells into odontoblasts of a "polarized or functional type, the latter cells then lay down dentine which, while building up, is calcified and is responsible for the growth of the tooth In the normal growth of the rat's incisor one may then expect an orderly sequence as follows 1 Proliferation of ameloblasts 2 Differentiation of ameloblasts 3 Differentiation of odontoblasts 4 Formation of dentine matrix 5 Calcification of dentine and enamel

ministered, the bones become extremely fragile so that numerous fractures result *Bone growth is greatly accelerated* so that "it is possible to get the equivalent of a year's growth in a ten to fifteen day period" (329) Wolbach interprets this action of vitamin A as an acceleration of "remodeling sequences in conformity to normal growth pattern The remodeling takes place in spite of a retardation of linear growth of bone and is correlated with accelerated epiphyseal cartilage sequences Those sequences retarded or suppressed in vitamin A deficiency are grotesquely and dramatically accelerated by the excess" (329)

Besides the cessation of bone growth which occurs as a result of vitamin A deficiency certain other osseous changes are encountered Mellanby (327) who has interpreted virtually all the neurological changes on the basis of bony overgrowth, has described hyperostoses about the periotic labyrinth and certain of the foramina of the skull in dogs Wolbach and Bessey (328) note such changes in the inner ear, narrowing of the optic foramina has been observed in calves (330) The significance and cause of these localized outgrowths of bone are not clear and all require further investigation

From the studies of the nervous tissues in rats it is to be expected that an increase in intracranial pressure occurs when these and other animals are placed *on vitamin A-deficient diets In deficient calves a quantitative rise* in cerebrospinal fluid pressure has been observed (331, 332) Ophthalmoscopic examination may reveal papilledema Cerebrospinal fluid values may rise from the normal of 70 millimeters of water to as high as 240 millimeters A further manifestation of increased intracranial pressure in such animals is the occurrence of cysts in the hypophysis which are not encountered in normal animals, they are observed in the posterior lobe and cause compression of the gland which is followed by atrophy and necrosis Measurements of the cysts have not been given, one, however, is said to have contained 75 milliliters of fluid (333) In the same species blindness has been observed but it is not entirely clear how much of a role each of these factors play increased intracranial pressure, narrowing of the optic foramina by bony overgrowth and degeneration of the retina

Teeth Because of the epithelial origin of the teeth it is not surprising to find that deficiency of vitamin A profoundly affects their growth The studies reported in rats and guinea pigs are all in agreement and the underlying changes appear to be well established (334, 335, 336) Before describing the alterations which occur in the rat's incisor it would seem advantageous to review briefly the normal development of this structure (336) Growth of the rat's incisor results from the proliferation of a group of epithelial cells at the base of the tooth Such odontogenic epithelium differentiates into ameloblasts which form enamel on the outer or labial surface of the tooth and cemetoblasts which form the cementum which is deposited



FIGURE 26 Lower incisor tooth for a vitamin A deficient rat There is a wide band of dentin under the enamel (empty space) A characteristic proliferation of odontoblasts is also seen (Courtesy of Dr Paul E Boyle)

on the lingual and lateral margins Odontogenic epithelium is also responsible for the organization of mesenchymal pulp cells into odontoblasts of a "polarized" or functional type, the latter cells then lay down dentine which while building up is calcified and is responsible for the growth of the tooth In the normal growth of the rat's incisor one may then expect an orderly sequence as follows 1 Proliferation of ameloblasts 2 Differentiation of ameloblasts 3 Differentiation of odontoblasts 4 Formation of dentine matrix 5 Calcification of dentine and enamel

In vitamin A-deficient animals the first and principle change is found in the odontoblasts It will be remembered, however, that such cells are organized by the odontogenic epithelium, hence, although in the early stages of the deficiency the latter cells are morphologically not abnormal, the physiological stimulus they ordinarily provide appears to be inadequate The odontoblasts do not differentiate or arrange themselves in normal fashion and in consequence dentine is formed irregularly and in varying amounts The lingual dentine is thin while that deposited over the labial surface is thicker than normal It has been suggested that masticatory stresses may lead to this difference (336) The odontoblasts show varying degrees of development, being more poorly differentiated proximally than distally, possibly because the former cells are younger and more deficient than those which are more distal and therefore, older

In the early stages of vitamin A-deficiency the odontogenic epithelium appears to be normal morphologically though not so physiologically, later, profound anatomical changes are observed The cells exhibit such a lack of differentiation that virtually no recognizable ameloblasts can be found Consequently there is a great reduction in the deposition of enamel, as a result enamel hypoplasia is a prominent manifestation of advanced vitamin A deficiency Since the odontogenic epithelium does not stop its proliferative activity, cords of undifferentiated epithelium invade the pulpal tissues where they form nests of cells Some of these are able to stimulate the neighboring mesenchyme to abortive effects of dentine formation and in this fashion numerous concretions may be formed Loss of the yellow pigment of the incisor teeth of rats has been described when rations deficient in vitaman A are employed (429)

In the rat and guinea pig all of the above changes are reversible following adequate treatment with vitamin A (334, 336)

In summary, vitamin A deficiency leads to changes in many epithelial tissues where metaplastic keratinization occurs In addition there are physiological and morphological disturbances in the retina and derangement in the growth of bones and teeth

Vitamin A Deficiency in Man Both physiological and morphological evidence of vitamin A deficiency may be detected in man In the United States, however clinical evidence of this deficiency is uncommon and alterations in the tissues at autopsy are rarely seen On the other hand in the Orient particularly in China (338), the clinical manifestations of vitamin A deficiency are much more common, here, however, the syndrome which is observed is not usually caused by a deficiency of vitamin A alone but of other essential nutrients as well

Many attempts have been made to place the diagnosis of vitamin A deficiency on an evaluation of the levels of carotene and/or vitamin A in the

blood serum It has become apparent, however, that the levels considered normal or subnormal in this country must be revised in view of data which have been accumulated in China (338) where blood levels have been correlated with the clinical manifestations of the disease and indicate that the levels used to delineate vitamin A deficiency in this country are too high

The characteristic clinical signs of vitamin A deficiency are said to include xerosis keratomalacia, nyctalopia, and a papular dermatitis The xerosis or hyperkeratosis of the conjunctivae, has the same appearance microscopically as similar changes in these tissues of experimental animals that is, keratinizing metaplasia Identical changes occur in the corneal epithelium which are followed by vascularization inflammation, and sometimes perforation Nyctalopia at one time was said to be common in this country but there is now a growing feeling that night blindness resulting from vitamin A-deficient diets is uncommon and that there is very little relation between the vitamin A content of the diet, the blood concentration of this vitamin and dark adaptation (339) The skin changes which have been thought to be specific for vitamin A deficiency have been discussed above where the conclusion is reached that the papullar dermatitis which has been thought to be pathognomonic results from a deficiency of not only vitamin A but portions of the B complex as well (324)

Several trials have been made to elicit uncomplicated vitamin A deficiency in experimental subjects The characteristic clinical manifestations of this deficient state have not been produced however even though such volunteers have remained on a vitamin A-deficient regimen for fairly long periods of time (340)

At autopsies performed in this country the manifestations of vitamin A deficiency are far more common in children than in adults Blackfan and Wolbach (341) have described the postmortem findings in a group of children whose clinical course was characterized by severe respiratory infections Typical keratinizing metaplasia was found in the kidney pelves bladder, the lining of the nasal sinuses the respiratory tract and in other tissues similar to those affected in experimental animals Of particular interest were the pulmonary lesions which consisted of bronchitis bronchiolitis and lobular pneumonia all of which were extreme It was apparent that in the absence of normal ciliated epithelium bacteria were not disposed of in the usual fashion and were able to grow invade the bronchial walls and produce inflammation of the surrounding structures Since a number of cases of vitamin A deficiency studied by these and other investigators have revealed cystic fibrosis of the pancreas or biliary obstruction it is concluded that absence of the pancreatic or hepatic secretions leads to poor or inadequate vitamin A absorption

Changes similar to those observed in the enamel organ of the rat have

been described in this structure of an infant exhibiting other manifestations of vitamin A deficiency, but since the general incidence of vitamin A deprivation in children is so low, it is unlikely that a deficiency of this nutrient is ever a common cause of enamel hypoplasia in the human (342).

At autopsy, morphological evidence of vitamin A deficiency in the adult is much more uncommon than in children. In fact, aside from the clinical manifestations referred to above, instances of clear-cut examples of keratinizing metaplasia in the adult are virtually non-existent except for the occurrence of xerosis in the eye. It should be pointed out that several years ago based upon observations in animals and little else vitamin A deficiency was introduced as a prominent cause of urinary calculi in man. Studies by Jewett et al. (343) who utilized the dark adaptation technique and whose material was examined by the present writer would seem to show conclusively that vitamin A deficiency is a rare cause of urinary calculi in man in this country at least.

Vitamins D

Historical. Although manifestations of rickets have been recognized from earliest times not until the middle of the 17th century was the disease shown to be a clinical entity. Pathological studies of the bones of animals and humans were made during the last century, such observations culminated in the morphological investigations of Pommer which were published in 1885 (345). Pommer outlined the broad principles of the pathological changes in rickets. Subsequent workers have only confirmed and amplified his conclusions.

Until the end of the second decade of the present century rickets had not been consistently produced in the laboratory. In 1918 Mellanby (346) announced from England that "Rickets is a condition primarily due to the lack of an accessory factor in the diet." Further studies (347) published several years later showed that cod liver oil, but not certain other fats has antirachitic effects, and that environmental conditions such as close confinement, appear to contribute to the development of the changes in the skeleton. Mellanby's rations were not adequate in other respects particularly fat-soluble vitamin A. In addition, the diagnosis of rickets was based in large part on the gross appearance of his dogs not on histological observations of the bones.

At the same time similar experiments were being carried out in this country, here it was conclusively demonstrated how important the calcium and phosphorus content of the diet is in relation to the development of bone changes. In 1921 Sherman and Pappenheimer (348) announced the production of rickets in rats by a diet deficient in phosphorus. The addition of phosphate protected against the development of skeletal changes. Similar

experiments were reported simultaneously by McCollum Simmonds Shipley, and Park (349, 350) who, employing a variety of diets, produced bone changes which were proved to be rickets by microscopic examination Such studies similarly pointed to the importance of the phosphate content of the diet, and further showed that lack of fat-soluble vitamin A seemed to be another important factor in the pathogenesis of the disease The Johns Hopkins investigators then went on to demonstrate that the inclusion of cod liver oil in the diet leads to the deposition of lime salts in the bones of rachitic rats (351) and that cod liver oil contains a curative substance not present in butter fat The existence of two fat-soluble vitamins was demonstrated when oxygen was bubbled through cod liver oil a procedure which destroys vitamin A while the antirachitic potency remains intact (352) Lastly the clinical efficacy of cod liver oil was proved for when this material was administered to rachitic children roentgenograms and histological studies clearly indicated that lime salts had been deposited in the bones (353)

While these studies were going on attention was also centered upon Huldschinsky's (354) observation that ultra-violet light has a curative effect on clinical rickets The entire story was brought to a close when Steenbock (355) and Hess (356) simultaneously showed that when various substances were irradiated antirachitic properties appeared The precursors of these active materials were demonstrated to be ergosterol and cholesterol

Biochemical Relationships Of the many forms of vitamin D which have now been discovered (357) two activated ergosterol (viosterol or calciferol) and activated cholesterol (7-dehydro cholesterol), are the most important, both are used extensively in the prophylaxis and therapy of rickets Aside from these dietary forms of vitamin D, the organism is able to obtain adequate amounts of antirachitic substance from the activation by sunlight of the pro-vitamin in the skin Hess and Weinstock (358) proved this by feeding human or calf skin to rats on a rachitogenic diet While non-irradiated skin has little or no healing effect, dermal tissues which have been radiated with ultra-violet rays *in vitro* do have antirachitic power These observations show conclusively why ultra-violet irradiation is so important in the cure and prophylaxis of rickets and also help explain the geographical distribution of the disease

Efforts to elucidate the mode of action of vitamin D have been directed at its general role in calcium and phosphorus metabolism Evidence has been adduced that vitamin D enhances the absorption of calcium from the intestinal tract (359) On the other hand, phosphorus absorption appears to be unaffected by the presence or absence of the vitamin (360) Utilizing radioactive calcium and phosphorus such observations have been repeated and confirmed (361 362)

Evidence for a local or tissue effect of vitamin D on bone is less con-

clusive Although it has been postulated that this vitamin promotes the conversion of inorganic phosphorus to organic forms in the bone (362, 363), more data are necessary before any final conclusions can be drawn

Normal Bone Growth In order that the pathologic changes which take place in rickets and in scurvy too, will be more intelligible a brief discussion of normal osteogenesis will be presented the anatomical aspects first, followed by pertinent data on the composition of bone and what little is known of its morphogenic physiology

Histogenically two types of bone are recognized membranous and endochondral (364) The former is found in certain portions of the skull but concerns us little since it comprises only a small part of the total skeletal system It suffices to say that in the formation of this type of bone, lime salts that is calcium and phosphorus, are deposited in sheets of connective tissue From the second type, endochondral bone, develop the bony tissues of the extremities, the ribs certain bones at the base of the skull, et cetera To epitomize the formation of this type, lime salts are first deposited in the matrices of cartilaginous plates Blood vessels then grow into these plates and begin to erode the cartilage cells, osteoblasts follow and deposit osteoid on the now calcified cartilaginous matrix Similar material which is rapidly turned into bone is deposited about the periphery of such cartilage plates to form the future shaft and to allow the structure to increase in width

Growth in length of the bone is brought about by a continuous proliferation and maturation of cartilage cells which make up the epiphyseal cartilage at the ends of the bony shaft The cells come to be arranged in parallel rows at the cartilage shaft junction and as they mature such cells grow larger and larger It is assumed that the lowermost ones die, thus becoming ready for invasion by capillaries from the shaft Coincident with these changes in the cartilage cells, lime salts are deposited in the matrix substance between them The tissue is thus transformed into a sort of honeycomb into which the capillaries grow The blood vessels are accompanied by ostoblasts which deposit the organic portion of bone osteoid, on the spicules of calcified cartilaginous matrix Simultaneously inorganic salts of calcium and phosphorus are deposited in this organic matrix Bone therefore, is composed of an organic protein of collagenous-like material and inorganic salts Bone deposited in this fashion at the cartilage shaft junction is soon destroyed in order to lighten the structure So too the shaft is continuously remodeled and growth in width keeps pace with growth in length as a result of new formation on the outer surface of the cortex and destruction along the inner margins In the human bone growth is most active during infancy After 2 to 3 years of age growth of the cartilage slows down tremendously, remodeling sequences are likewise reduced in the shaft However it must be clearly understood that even in the adult bone is not a static or "dead"



FIGURE 27. Normal bone. The costo-chondral junction from a six months-old colored boy dying acutely of bacillary dysentery. Note small undifferentiated cartilage cells lying in an haphazard fashion at top. These are arranged in rows and become larger at the straight and even cartilage shaft junction. Here they are being invaded by capillaries. The dark staining material between the rows is calcified cartilaginous matrix upon which, as the capillaries destroy the cartilage cells, osteoblasts deposit osteoid which is instantaneously converted into bone by the deposition of lime salts in it. Note presence of calcified matrix in some of the bony trabeculae deeper in the shaft. The marrow elements are found close to the cartilage shaft junction, a normal phenomenon. H. and E., ×15.

tissue. Quite the contrary, bone is being continually destroyed and rebuilt, such metabolic activity is pronounced as can be demonstrated with radioactive isotopes of calcium and phosphorus.

As noted above, bone consists of inorganic and organic materials which are intimately related. The organic portion is mainly protein in nature. Calcium and phosphorus comprise the greater portion of the inorganic constituents of bone. In addition, however, there are appreciable quantities of sodium, magnesium, potassium, carbonate, citrate, fluoride, sulfate, chloride as well as other elements. Since the amounts of these materials vary from specimen to specimen, it is quite clear that the structure of bone is determined by the blood concentrations of its various constituents. The calcium and phosphorus content is fairly constant, however, being in the order of 2 to 1. The precise chemical composition of bone is complex and is not

clusive. Although it has been postulated that this vitamin promotes the conversion of inorganic phosphorus to organic forms in the bone (362, 363), more data are necessary before any final conclusions can be drawn.

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although it has obvious limitations, has been very useful in studies of bone physiology. Broadly speaking, when the product of calcium and phosphate is under 30, lime salts will fail to be deposited; above 40, they will be laid down in normal fashion. This importance of the concentrations of calcium and phosphorus has been amply demonstrated *in vitro*, for when slabs of bone from rachitic rats are placed in solutions containing optimum concentrations



FIGURE 29 Normal bone. The cartilage shaft junction of a rib from an older child, an eight year old white female. Note that there are fewer trabeculae at the cartilage shaft junction and those which persist are short and squat. The zone of proliferative cartilage cells is narrower than that in Figure 27. Fat is partially replacing myeloid elements in the marrow spaces. A comparison of this bone with that of the six-months-old child shown in Figure 27 should bring out the reasons why certain of the nutritional diseases of bone are not seen in the older child, that is when growth becomes so slow. H and E ×15

of calcium and phosphorus, lime salts are deposited in the rachitic cartilage (367, 368), but when the calcium and phosphorus contents are not optimal, these salts do not deposit.

A local factor must also be invoked to explain the deposition of lime salts in bone since their concentrations in plasma will not account for chemical precipitation. The following theory proposed by Gutman is current at this writing (369, 370). It is well known that there is an abundance of glycogen in mature proliferating cartilage cells (371). A phosphorylase has been demonstrated in epiphyseal cartilage which appears to transform this glyco-

entirely settled as yet Based in the main on x-ray crystal diffraction studies, bone has been likened to a salt of the apatite series, composed of crystals in lattice arrangement on which the other inorganic compounds are adsorbed The most widely held hypothesis at present is that calcium and phosphate ions combine, due to factors to be discussed below, to form $CaHPO_4$ and that this is transformed into $Ca_3(PO_4)_2$ The other constituents are then



FIGURE 28 Normal Bone Higher power of same section shown in Figure 27 The arrangement of the cartilage cells is more clearly seen So too the calcified matrix material and the deposition of bone upon it are more readily appreciated than in the lower power H and L ×60

adsorbed on this framework The final formula is usually written, $CaX \cdot n\ Ca_3(PO_4)_2$ where X represents the various acidic radicals mainly CO_3 and *n* is 2 or 3 (365)

Although the histogenesis of bone is clear enough and its composition is also fairly well established, the chemistry of bone formation has yet to be completely elucidated and *is unique in that it does not appear to obey any* of the rules of physical chemistry The biochemistry of bone salt deposition is thought to be governed by two main factors general and local The first deals with the concentrations of calcium and phosphorus in the blood plasma a concept which was first elucidated by Howland and Kramer (366) and

counterpart of rickets osteoblastic activity is not affected unless the organism is suffering from inanition or some intercurrent disease Therefore as one would expect, osteoid which is the organic matrix of bone is deposited upon pre existing bony trabeculae in normal fashion However since general and perhaps local factors are not propitious calcium and phosphate salts are not



FIGURE 30 Shaft of Rib Moderate Rickets This is the shaft of the same section shown in Figure 32 and shows several trabeculae which are coated by broad zones of osteoid which stands out as a paler tissue than the bone which it encases Note that the width of this coating of osteoid about a given trabecula is not uniform in thickness H and E ×60

deposited in this osteoid It will be recalled that ordinarily the deposition of inorganic salts occurs virtually simultaneously with the deposition of osteoid in rickets however deposition of lime salts is either retarded or completely lacking In the usual tissue sections stained with hematoxylin and eosin osteoid may be recognized as a band of light pink-staining material of varying width which coats portions of the cortex and trabeculae of the shaft In such histological preparations bone usually has a bluish-gray tint The contrast between bone and osteoid may be accentuated if more elegant techniques are used such as that of McLean and Bloom (364) who employ undecalcified sections stained with silver nitrate When studying any bone

gen to glucose-1-phosphate, which ester is then transformed by a phospho glucomutase *into glucose-6-phosphate* The latter, together with some of the glucose-1-phosphate is then broken down by a phosphatase (372) which can be demonstrated to be present by histochemical techniques The increase in local concentration of inorganic phosphate which is thus produced leads to a precipitation of calcium phosphate or a similar compound in the cartilaginous matrix substance *It must be stressed that this mechanism can only* function in one of two ways to produce a sudden outpouring of phosphate which will lead to a large excess at a given moment or to furnish energy which may be necessary to pull calcium and phosphorus too from the blood stream Hydrogen ion concentration may also be a factor although the methods which have been employed to study this such as dyes (373) and quinhydrone electrode (374) give no definite confirmation With the latter method which is not claimed to represent the true hydrogen ion concentration, average values of 7 35 for resting normal cartilage and 7 39 for proliferating *cartilage have been obtained* *It should be pointed out that this* local theory tends to explain the deposition of lime salts at the cartilage shaft junction, the mechanism in the shaft is more obscure

Pathological Effects Manifestations of vitamin D deficiency and/or associated deficiencies in calcium and/or phosphorus occur endemically or have been produced experimentally in a large variety of Mammalia It is not necessary to mention all the various species which have been studied since Goldblatt s review (375) may be consulted The common laboratory animal used for the investigation of experimental rickets is of course the rat In the discussion which follows we shall draw upon the literature (345, 376 377 378 379) as well as our own observations (115) of rickets in the rat and in particular, a large series of children aged from birth to fourteen years coming to autopsy at the Johns Hopkins Hospital This latter material has been studied in association with Dr E A Park and Miss Deborah Jackson

The manifestations of vitamin D deficiency or rickets are found only in the bones and teeth Changes which occur in other tissues are entirely secondary to alterations in the former structures

Bones The morphological criteria of rickets are found in the bony shaft as well as at the cartilage-shaft junction The degree of change which will be encountered in the latter site is dependent on the rate of growth of the bone which is especially dependent on the age of the organism The changes which occur in the shaft are straightforward and will be discussed first In the normal growth of the trabeculae and cortex constant remodeling sequences are taking place in the shaft Trabeculae are being continually destroyed and rebuilt likewise the cortex particularly when growth in diameter is taking place, is destroyed along its inner margin and built up along its external surface In rickets and in osteomalacia which is the adult

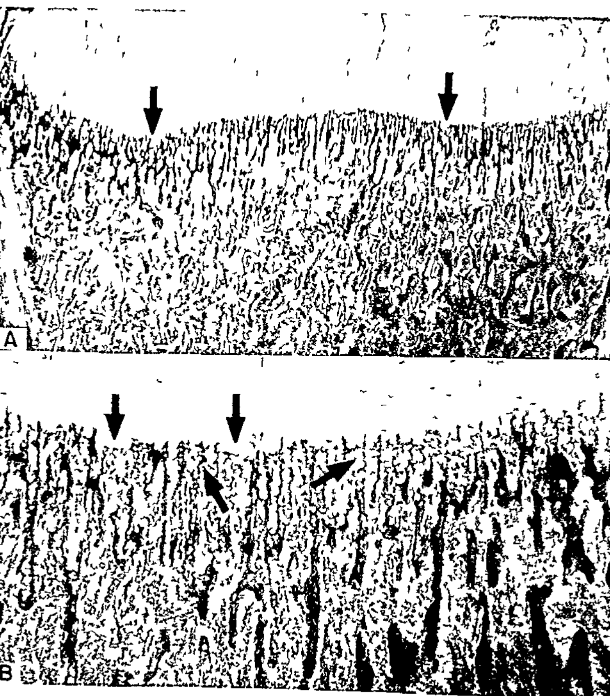


FIGURE 31 Costo chondral Junction Early Rickets *A* Cartilage shaft junction from a six months-old white female dying of staphylococcal sepsis The line of ossification is even and regular The only change which one can detect under this power is a diminution in the amount of lime salt deposition in the cartilaginous matrix Definite defects can be made out at arrows H and E., x15 *B* Higher magnification (x60) of same section shown in A The defects in calcification are more clearly shown There is no apparent excess of osteoid although deeper in the shaft osteoid is present in greater quantities than normal Note also that the spicules of calcified matrix are beginning to buckle under the strain of weight bearing (arrows) Compare with the normal in Figure 28 page 108

one should always examine the shaft to ascertain whether osteoid is present in abnormal amounts. One must, of course, bear in mind that the amount of normal or physiological osteoid varies depending upon the age and the species from which the specimen is derived. In the normal growing rat, for instance, osteoid is virtually never seen. In the new-born infant, especially the premature baby, osteoid is usually present and is especially prominent along the inner margins of the cortex. Osteoid is not ordinarily encountered in older children (after 2 years) and in adults, if present in these, it denotes rickets or osteomalacia. In rickets and osteomalacia osteoid borders of uniform thickness do not cover each and every trabecula, on the contrary the *deposition of osteoid is usually irregular and is doubtless related to mechanical* stress and strains. The absence of osteoid does not connote that rickets is not present, changes at the cartilage shaft junction may be recognized in certain instances in which osteoblastic activity *in the shaft* is so reduced as by wasting disease for instance that little or no osteoid is deposited.

Changes at the cartilage shaft junction in rickets may be epitomized as follows. 1) failure of lime salts to be deposited in the cartilaginous matrix material, and 2) failure of cartilage cells to undergo degeneration so that capillaries are unable to penetrate the cartilage except in a very irregular fashion.

In normal growth of the cartilage plate on the shaft, the cartilage cells multiply and those nearest the diaphysis arrange themselves in rows with the largest and most adult cells nearest the capillaries advancing from the shaft. *Lime salts are deposited in the cartilaginous matrix substance between the* rows of hypertrophic cartilage cells, this deposit seems to guide the ingrowth of capillaries into the holes left by the degenerating cartilage cells. Osteoblasts then form osteoid on these spicules of calcified matrix. In rickets the initial change at the cartilage shaft junction is failure of lime salts to be deposited in the cartilaginous matrix substance. Such defects are exhibited in sections by an absence of deep blue-staining material (calcium salts) between the rows of cartilage cells. The extent of such defects is dependent upon the severity of the metabolic disorder.

Coincident with this defective lime salt deposition the zone of mature *cartilage cells begins to increase in width* which is interpreted to mean that such cells are more resistant to destruction than normal. The reason for this is not clear but may be a local result of vitamin D deficiency or may be related to the disturbance in blood calcium and phosphorus relationships. More likely however it is dependent on mechanical factors accompanying the lack of support usually supplied by the calcified matrix substance for there is compression and swelling of the cartilage cells nearest the shaft which may prevent the ingrow*th of capillaries*. In any event the cartilage ceases to be invaded or is destroyed in a very irregular fashion.

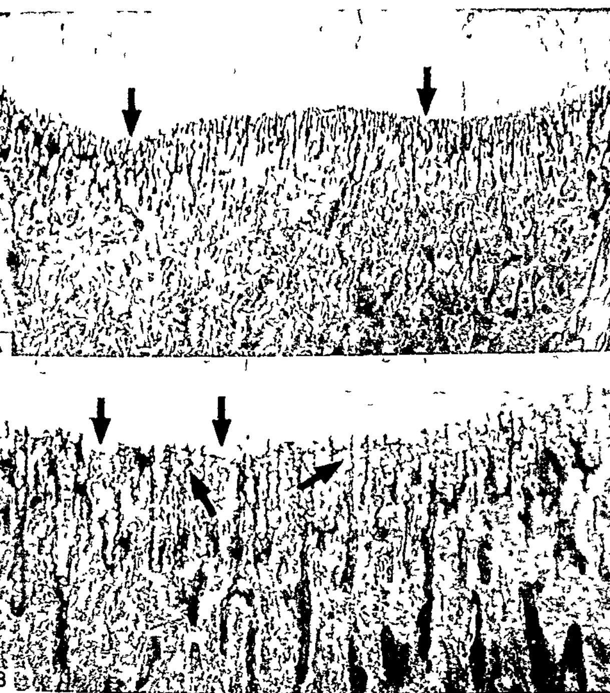


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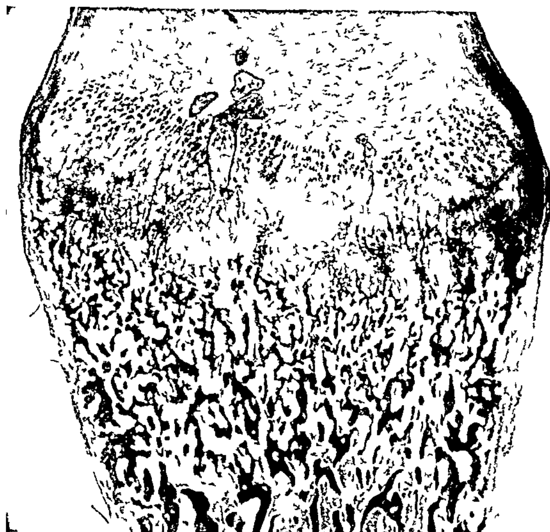


FIGURE 37 Rib Moderate Rickets Costochondral junction from a seven month-old colored male dying acutely in three days of lobular pneumonia Note increase in width of cartilage shaft junction Especially prominent in the increase is width of zone of proliferative cartilage cells and irregularities in the calcification of this region Note also tongues of cartilage projecting down toward the shaft surrounded on either side by invading vessels The trabeculae beneath the cartilage are more numerous than usual There is a great deal of osteoid on such trabeculae this is shown in Figure 30 which is a higher power of this section H and E x15

When the disease has progressed for a time, the histological picture is characterized by a broad zone between the multiplying cartilage cells and the shaft the so-called rachitic metaphysis This is composed of tongues of cartilage which extend down toward the shaft and which are separated from one another by collections of capillaries or vascular bushes In other words at some point blood vessels have been able to penetrate the cartilage while in other situations due perhaps to compression of cartilage or differences in lime salt deposition capillaries find it impossible to erode the cartilage In addition this zone contains trabeculae made up of uncalcified cartilage matrix upon which osteoid is being deposited These of course result from

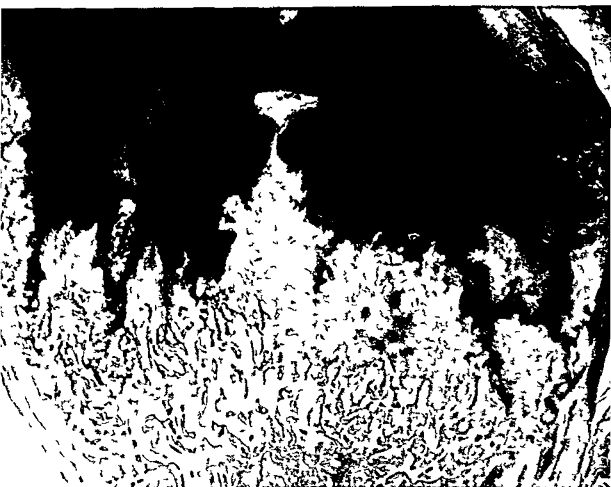


FIGURE 35 Rib Severe Rickets Costochondral junction from a seven months old colored male dying of unexplained fever and diarrhea he had been sick for one month There is extreme swelling in the region of the cartilage shaft junction Note as in Figure 32 the increase in width of the zone of mature cartilage cells and the irregularities in calcification There is complete disorganization in this region due to collapse of the cartilage and trabeculae many of which are composed of osteoid H and E., × 15

groups of cartilage cells being cut off by capillaries and then coated with osteoid doubtless an attempt to strengthen this region The rachitic metaphysis manifests itself clinically as a swelling or beading of the ribs or increase in width of the ends of the long bones of the extremities

When therapy is instituted repair occurs, the initial change at the cartilage shaft junction is the deposition of lime salts in the cartilage adjacent to the rachitic metaphysis Lime salts are also deposited in various parts of the swollen cartilage shaft junction and in the shaft which finally results in calcification of these portions The entire area is ultimately remodeled Deformities of course, in the form of bending of the long bones may exist for life It is of interest that there is some evidence in rats that the rachitic pattern as evidenced by X-ray diffraction studies remains for some time and may never be completely eradicated (383)



FIGURE 54 Rib Healing Rickets Costochondral junction from a 19 months-old white baby dying of a lung abscess and empyema after being sick for six weeks There is little if any swelling at the cartilage shaft junction The only evidence of healing rickets is found in the cartilage The zone of hypertrophic proliferative cells is increased in width More striking however is the presence of a line of dark staining material in the cartilage above the cartilage shaft junction This zone is composed of calcified cartilaginous material and represents a resumption of the deposition of lime salts at the place where they should have been laid down had rickets not been present H and E x15

Teeth Changes in the teeth in rickets are less complex than those occurring in bones mainly for the reason that the former are never resorbed When young rats are placed on a rachitogenic diet (high calcium, low phosphorus, no vitamin D), the first and most prominent change is in the incisors where a line of disturbed calcification appears in the dentine, this has been called the 'calciotraumatic line" (380) It is found in the dentine and represents the first response of the organism to the effects of the rachitogenic regimen Almost immediately too, there is a retardation in the formation of predentine together with a pronounced disturbance in the calcification of all the dentine which is formed, this material is not homogeneously basophilic but is stippled by an irregular deposition of calcium salts Calcification of the cementum is likewise retarded The changes in the molars are similar but not of such a severe degree Although there are cystic alterations in the enamel organ before it undergoes atrophy no other abnormalities in this structure can be detected There is no enamel hypoplasia in rats although

in the guinea pig severe hypoplasia of the enamel has been reported (382) when these animals are placed on a low calcium-high phosphorus diet containing no vitamin D. Thus in experimental animals there does not appear to be entire agreement, though it will be noted the calcium and phosphorus concentrations of the diet were reversed and this may explain the presence or absence of enamel hypoplasia. Pointed studies on the teeth using diets of known composition while changing the calcium and phosphorus ratios and total concentrations as employed by Shohl (377) in the study of bone are certainly needed.

As might be expected, the bony supporting structures of the teeth show characteristic changes similar to those of the bones just described above. Wide osteoid borders are found on the trabeculae of the alveolar bone (381).

Rickets in the Human. Since the metabolism of calcium, phosphorus, and vitamin D are so closely related, the heading of this section is Rickets rather than Vitamin D Deficiency. It is obvious that the manifestations of rickets in the skeleton may be produced by a deficiency of calcium, phosphorus or vitamin D singly or together. Students of nutrition seem to be agreed that calcium deficiency is prevalent in childhood and adolescence. Inasmuch as the geographical incidence of rickets still seems to prevail, it is apparent that adequate amounts of vitamin D are not ingested as well. In a given case of rickets however it is usually difficult to determine which of many factors was the forerunner of the metabolic disturbances which culminated in the abnormal changes in the skeleton.

The use of clinical and X-ray evidence in determining the incidence of rickets in the population at large is not satisfactory, nor is the biological estimation of vitamin D in the blood practically feasible (389), such data are much inferior to an histological examination of the skeleton. A study of the latter type was carried out by Schmorl during the years 1901-1908 in Dresden (384). Rickets was found to be present in 61 percent of all children dying during the third month of life, in 94 to 98 percent during the fourth to eighteenth months, and in 91 per cent from the nineteenth month to the beginning of the third year. A similar investigation has been made of all children dying in the Johns Hopkins Hospital during the period 1928-1942. Pathological criteria of rickets were found in 48.4 percent of a group of children aged 3 to 19 months (385). In the age group 2 to 14 years microscopic evidence of rickets was present in 46.5 percent (386). Inasmuch as moderate and severe rickets was found in many children dying of acute disease, it is valid to infer that at the latter age "rickets is of frequent occurrence in healthy appearing children." Conclusions should be withheld until similar histologic studies are made in other parts of the United States and the world in order to assess the distribution of rickets and correlate its incidence with dietary and geographical factors. The clinical aspects of rickets will not be



FIGURE 34 Rib Healing Rickets Costochondral junction from a 19 months-old white baby dying of a lung abscess and empyema after being sick for six weeks There is little if any swelling at the cartilage shaft junction The only evidence of healing rickets is found in the cartilage The zone of hypertrophic proliferative cells is increased in width More striking however is the presence of a line of dark staining material in the cartilage above the cartilage shaft junction This zone is composed of calcified cartilaginous material and represents a resumption of the deposition of lime salts at the place where they should have been laid down had rickets not been present H and E x15

Teeth Changes in the teeth in rickets are less complex than those occurring in bones mainly for the reason that the former are never resorbed When young rats are placed on a rachitogenic diet (high calcium, low phosphorus, no vitamin D), the first and most prominent change is in the incisors where a line of disturbed calcification appears in the dentine, this has been called the 'calciotraumatic line" (380) It is found in the dentine and represents the first response of the organism to the effects of the rachitogenic regimen Almost immediately, too, there is a retardation in the formation of predentine together with a pronounced disturbance in the calcification of all the dentine which is formed, this material is not homogeneously basophilic but is stippled by an irregular deposition of calcium salts Calcification of the cementum is likewise retarded The changes in the molars are similar but not of such a severe degree Although there are cystic alterations in the enamel organ before it undergoes atrophy, no other abnormalities in this structure can be detected There is no enamel hypoplasia in rats although

disease Non-dietary osteomalacia may be encountered in this country at autopsy, for instance, the present writer has found widened seams of osteoid along the trabeculae of the vertebra in about half of a group of adults dying of chronic renal insufficiency (388) Such changes are, of course, partially explained by the deranged calcium and phosphorus metabolism which occurs in chronic nephritis

The relation of calcium phosphorus and vitamin D to the structure of the human tooth and to dental caries in particular is not too clearly understood So many factors, such as carbohydrate, mouth flora fluorine to name only a few, play a role in the production of caries that the effects of the 3 nutrients cited above and especially vitamin D seem almost impossible to determine Inasmuch as this book deals primarily with pathological effects produced by deficiencies of single nutrients and since the subject of dental caries is so controversial it seems unwise to enter a field in which there is so much disagreement When such is the case it is evident that the data are sufficient not to convince but to confuse everyone

Vitamins E

(Alpha-tocopherol and its Homologs)

Historical In 1922, Evans and Bishop (390) reported the existence of a new dietary factor which was found to be necessary in order to insure normal reproduction in rats, this "X" factor was considered to be distinct from the then known vitamins A B and C Mattill et al (391) soon showed that testicular degeneration occurred in rats whose diets were deficient in this material A third important manifestation of vitamin E deficiency (as the factor had by then been named) was reported by Evans and Burr (392) in 1928 when the development of paralysis in young rats of mothers on vitamin E depleted diets was described Ten years passed before Olcott (393) showed that this "paralysis" was not neurogenic in origin as had been thought but was due to necrosis of striated muscle fibers

During the period in which morphological changes in vitamin E deficient animals were being studied, work was pushed on the identification of an active principle In 1936 Evans and his group (394) announced the isolation of certain alcohols from wheat-germ oil, one of which had strong vitamin E-like properties This alcohol was named alpha-tocopherol (tokos = childbirth phero = to carry) Soon after Karrer and his associates (395) succeeded in synthesizing a biologically active product

Biochemical Relationships Very early in the studies of the chemical prop-

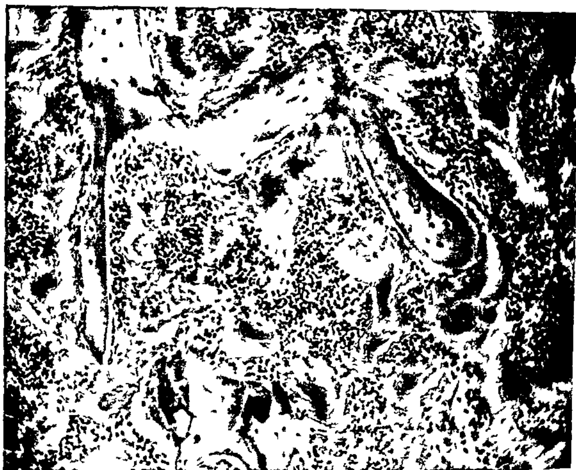


FIGURE 35 Shaft of Rib Rickets This is a section from a 12 year-old colored girl dying of acute meningococcal meningitis It is reproduced to show the bands of osteoid which are the only evidence of rickets to appear in the bone at this age since growth at the cartilage shaft junction has almost completely ceased Note as in Figure 30 the osteoid is irregularly dis tributed over the bony trabeculae H and E ×60

discussed here, its pathological manifestations in man were taken up in the preceding section The final outcome of these pathological changes is a variety of skeletal deformities genu varum and genu valgum, enlargement of the ends of the long bones, kyphosis deformity of the pelvis and deformity of the thorax The latter may be severe enough to lead to respiratory embarrassment (116)

Osteomalacia is the adult counterpart of rickets Although there have been no pointed studies of the prevalence of osteomalacia in this country, it is likely that this disease as a purely nutritional one is uncommon In China however, osteomalacia is said to be extremely prevalent although evidence for its incidence rests mainly upon clinical rather than pathological criteria Such cases, which usually occur in child-bearing women, are due to inadequate dietaries and insufficient sunlight Inasmuch as osteomalacia which has been described in North China (387) has a rather peculiar geographical distribution, one wonders whether any other factors might condition the

accompanied by poor development of the blood islands In addition irregular development of the liver is prominent together with an absence of blood cells in the heart and large vessels By the twelfth day the deficient embryo is a full day behind its control in development and on the thirteenth day death of the organism is apparent at which time the tissues become macerated

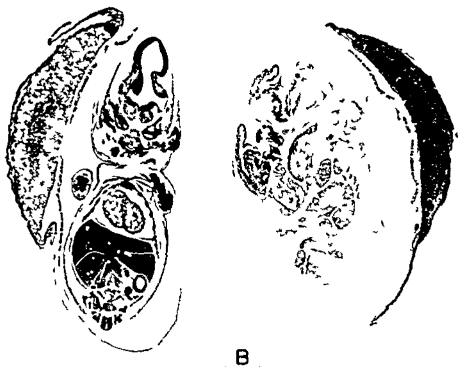


FIGURE 36 Reproduction Vitamin E Deficiency Two fetuses removed from females at the sixteenth day of pregnancy *A* from a normal and *B* from a vitamin E deficient female at the same gestation period The placenta of the latter is a little smaller than that of the normal In addition it is obvious that the fetus in *B* is dead and is undergoing extensive autolytic changes Note the differences in the color of the liver heart and cerebral tissues ×6 (Courtesy of Dr Karl E Mason)

and are resorbed Studies of the placenta indicate a marked retardation and an underdevelopment of vascular invasion by the fetal vessels Evans et al (402) have attempted to explain the reproductive failure on a starvation and asphyxia theory fetal nutrition is interfered with because of poor connections between fetal and maternal tissues and those nutrients which reach the embryo especially oxygen do not obtain proper distribution because of inadequate hematopoiesis Whether such an hypothesis is tenable remains to be settled by further investigation since Mason (406) has postulated a physiological or morphologic defect of the fetal blood vessels as the primary cause of the pathogenesis of the changes observed The young of female animals given borderline doses of vitamin E may show marked dilata-

erties of vitamin E it was recognized that the active principle had strong antioxidant properties As a result of studies of the oxidation of fat tissue the metabolism of muscle and the destruction of other nutrients *in vivo* current theories of the mode of action of alpha-tocopherol places its role as an antioxidant in the foreground

The body fat of rats which are raised on a diet deficient in alpha-tocopherol is very susceptible to oxidation (396), when the missing factor is administered body fat is stabilized and oxidation does not take place The relationship of alpha-tocopherol to muscle metabolism *in vitro* will be discussed in detail below It has been suggested that alpha-tocopherol "acts as a brake on the oxidative mechanism primarily of skeletal muscle and in its absence these oxidative processes in muscle run riot' (397) Studies of the interrelation of vitamin A and alpha-tocopherol have shown that the inclusion of the latter in a diet containing vitamin A prevents the destruction of vitamin A in the gastrointestinal tract (398) Somewhat similar experiments demonstrate that other fats such as cod liver oil destroy alpha-tocopherol and thus produce the pathological lesions characteristic of vitamin E deficiency (399)

Alpha-tocopherol is apparently absorbed like the other fat-soluble vitamins The importance of normal intestinal secretions particularly bile in absorption has been demonstrated in dogs with biliary fistulas (400) Alpha-tocopherol is not distributed in rat tissues as its fat-soluble properties might indicate For instance rather high concentrations may be demonstrated in heart spleen and lung although these tissues contain relatively little fat (401)

Pathological Effects Alpha tocopherol is necessary for the development of the embryo for the integrity of the male germinal epithelium and for the maintenance of the metabolism of striated muscle *in vitro* and structure *in vivo*

The indispensability of this vitamin in the reproductive process has been demonstrated in rats (402 403) mice (404) and guinea pigs (405), Evans and his co-workers (402) have studied this phase extensively in the first species where changes are found in the embryo and its membranes Ovulation the estrous cycle as well as ovarian and uterine tissues are all normal save for the presence of pigment which will be discussed below Female animals mate normally no microscopic evidence of damage to the ovum or its membranes can be detected until the middle portion of pregnancy that is at the time of implantation The earliest evidences of any untoward effects are found during the eighth day when an ectodermal cavity fails to appear Further evidence of deranged development occurs during the following days The ectoplacental and amniotic cavities fail to form as a result of inadequate growth of ectoderm and there is retarded development of fetal mesoderm

changes with liquifaction of chromatin and segregation of this material to form crescents at one side of this structure Changes in the cell membrane are postulated since such cells coalesce to form large characteristic multinucleated masses During this period degeneration of the primary spermatocytes and spermatogonia is observed Although some of the Sertoli cells degenerate for the most part these cells are not particularly damaged *The end result is a structure where tubules are atrophic and lined only by Sertoli syncytium* It is of great interest that if one testis of a vitamin E deficient rat is removed fairly early in the deficiency and examined it may appear perfectly normal under the microscope Nevertheless, even though more than adequate amounts of the missing nutrient are administered, the changes described in the end-stage above are found in the opposite one many days later In other words irreversable injury takes place even before it can be detected morphologically This of course is quite different from what occurs in vitamin A deficiency and inanition where repair can always be obtained (408)

It is now known that the 'nutritional muscular dystrophy' of rabbits and guinea pigs, which Goettsch and Pappenheimer (411) described in 1931, is due to a deficiency of alpha-tocopherol (412) Lesions have been adequately described in the skeletal musculature of the rabbit (411) guinea pig (412) young rat (413) and mouse (414)

Biochemical changes which will shortly be referred to occur in the muscle before any morphological criteria of damage appear (415) The initial histological abnormality consists of swelling and hyalinization of the muscle fiber which then becomes necrotic Sometimes there is an increase in the fluid content of the interstitial spaces and such fluid occasionally contains enough protein to be stained by the usual procedures Leukocytic *infiltration has been described as a prominent feature and there is marked* proliferation of the carcolemma nuclei An increase in fat globules likewise appears together with globules of a peculiar golden pigment, which will be discussed more fully below Many of the necrotic muscle fibers are infiltrated with calcium salts, which may be identified by appropriate stains Following the administration of alpha-tocopherol there is prompt regeneration of the damaged muscle fibers and an ultimate return of the tissue to its normal appearance in the rabbit and guinea pig Muscle lesions in the rat do not respond well to therapy Accompanying the degeneration of muscle fibers a disappearance in motor end plates has been demonstrated in the rat (417) The number of nerve endings returns to normal following repair of the dystrophic alterations Adequate data on the behavior of sensory endings are not available

As might be expected profound disturbances in the chemical composition and physiology of the muscle fibers accompany these morphological altera-

tion of the blood vessels and extensive hemorrhage into the tissues This is followed by death of the latter elements Mason suggests that the paucity of blood islands and cells upon which Evans places so much emphasis are secondary to loss into the dilated vascular channels

Degeneration of the male germinal epithelium has been described in the rat

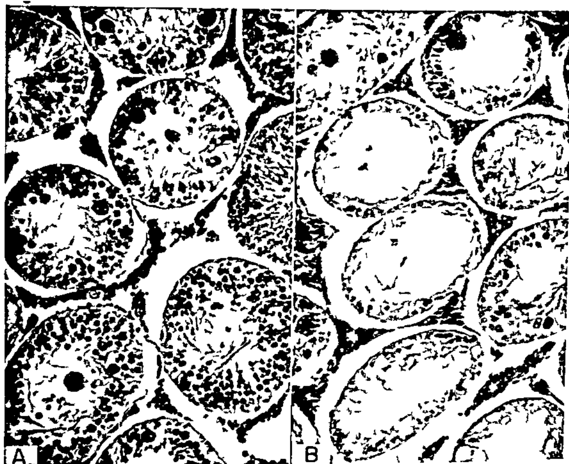


FIGURE 37 Testis Vitamin E Deficiency *A* and *B* Various stages in the development of testicular atrophy in animals placed on a vitamin E deficient diet Compare with Figure 1*A* There is an increasing diminution in the cells lining the tubules together with the appearance of characteristic giant cells which are especially prominent in *A* There is a decrease and virtual absence of spermatozoa spermatids and in some spermatocytes In *B* the same giant cells are seen but there is a further reduction in the number of germinal elements so that in several of the tubules only the Sertoli syncytial cells remain Eosin methylene blue stain ×150 (Courtesy of Dr Karl E Mason)

(407, 408), and guinea pig (409) but not in the rabbit (410) or mouse (404) although muscular lesions occur in these species Mason (407) divides the sequence of events in the rat into several stages as follows after fifty to one hundred days on the deficient diet there is chromotolysis and fusion of the mature spermatozoa, the debris then finds its way into the epididymis Following the disappearance of spermatozoa the spermatids assume a vesicular form and disintegrate Next the spermatocytes show peculiar nuclear

(418) In rabbits an increase in fat, phospholipid and cholesterol concentrations have been observed in the skeletal muscles and in addition there is an increase in the blood cholesterol levels (419) The creatine content of muscle is found to be reduced (420) and there is an increase in the excretion of this substance in the urine, so that the course of the syndrome and the response of the animal to therapy may be followed by studies of creatine excretion

By far the most interesting biochemical change in the muscle tissues of alphatocopherol depleted animals is a marked *in vitro* disturbance in respiration Increases in oxygen consumption of two hundred to four hundred percent were first described by Victor (421) The more recent studies of Houchin (397 422, 423) have furthered our knowledge of the metabolic phenomena The increase in QO_2 has been confirmed as well as the decrease in creatine content and high chloride concentration which have been described by others In the striated muscle from deficient animals of various species the oxygen consumption is as follows hamster, 240-250 percent of normal, rabbit 220 percent of normal, nursing rat, 160 percent of normal, grown rat 125 percent of normal Following the oral administration of alpha-tocopherol to deficient hamsters the QO_2 falls to normal levels in as short a time as twenty-two hours Studies of biopsied muscle tissues from depleted rabbits to which alpha-tocopherol phosphate had been given intravenously indicates that there is a drop in the QO_2 of 34 percent in the first hour and 49 percent more in the next three hours

In vitro observations of dystrophic muscle slices from rabbits and hamsters show that the addition of alpha-tocopherol to the medium lowers the QO_2 by 40 percent Muscles slices from normal animals are not so affected The succinoxidase activity of dystrophic hamster muscle is found to increase 160 percent above normal Addition of alpha-tocopherol to the medium decreases the succinate activity toward normal In view of these *in vitro* studies of muscle metabolism it is unfortunate that the data are so inadequate with regard to the total metabolism of the organism When a group of vitamin E deficient rats is compared with a similar group which had secured alpha-tocopherol on the 15th day of life the total oxygen consumption of the latter animals is lower Unfortunately however there were no very marked differences between the former group and stockfed controls (415) This is a subject requiring further investigation

Pappenheimer and Goettsch (424) made the interesting observation that complete denervation of an extremity prevents the development of muscular lesions in that limb The reason for this is not clear whether the abolition of motor or sensory or sympathetic impulses or all is responsible has yet to be determined

In rats which had been on a deficient diet for over twelve months necrosis of cardiac muscle fibers followed by fibrosis has been observed by Mason

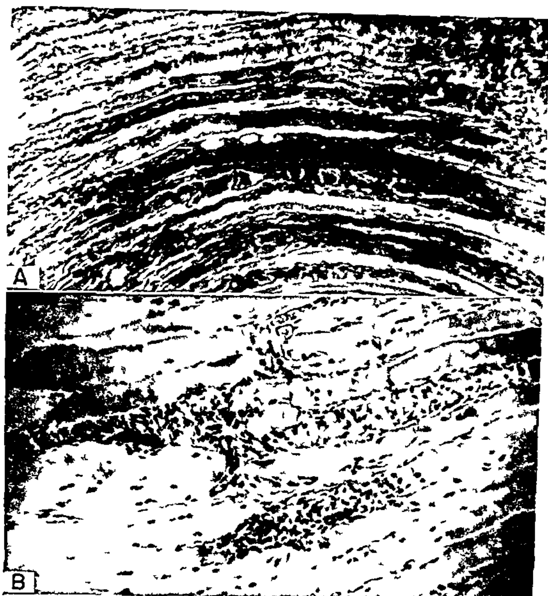


Figure 58 Striated Muscle Vitamin E Deficiency *A* Muscle from rabbit showing destruction of fibers with cellular infiltration and proliferation of sarcolemma nuclei In several places there are fat vacuoles H and E x130 *B* Hamster showing similar changes with destruction of muscle fibers and cellular infiltration In addition though not showing well in this photomicrograph there are macrophages filled with the acid fast pigment ceroid x150 (Courtesy of Dr Karl E Mason)

tions, in fact increased oxygen consumption of the muscle tissue occurs *in vitro* before any histological lesions can be demonstrated (415) In the rabbit (416) the potassium and magnesium contents of the muscle are found to be decreased while the concentrations of sodium and chloride are increased the former out of proportion to the latter The muscle content of calcium and phosphorus is increased in animals which show histological evidence of calcification In rats an increase in acid soluble phosphorus compounds and a reduction in the phosphorylation of glycogen has been noted

animals Small globules of pigment are found in the uterine muscle fibers ovary, interstitial cells of the testis, lymph nodes spleen, fat, macrophages of the liver, bone marrow, lung, kidney, as well as voluntary and cardiac muscle The pigment is acid-fast and apparently first accumulates in the uterine muscle It is readily taken up by macrophages, but these cells do not appear capable of digesting it Tocopherol treatment seems to arrest pigment production, but does not appreciably increase its rate of disappearance When 20 percent cod liver oil is incorporated in the diet of vitamin E-deficient rats, an intense brown discoloration of the adipose tissue appears and microscopic examination reveals even more pigment in the tissues mentioned above with particularly large accumulations in the fat (430) The pigment produced by vitamin E deficiency alone which is intensified by adding cod liver oil to the deficient diet resembles "ceroid" (page 000) in many respects

The yellow color of the rat's incisor is of course familiar to all who have worked with this species The pigment which contains iron is said not to be a porphyrin or lipochrome but perhaps a melanin-like material and is apparently deposited by the ameloblasts (429) The fat and vitamin E content of experimental rations have been shown to affect the pigmentation of this tooth When animals are maintained on diets devoid of fat and vitamin E, normal pigmentation occurs, however when twenty percent lard or cod liver oil are added to the ration the yellow color fails to appear The active portion of these two fats is apparently in the highly unsaturated fraction (431 432)

Several groups of investigators have described extensive lesions in the nervous tissues of vitamin E deficient animals Wolf and Pappenheimer (433) have critically reviewed this work and concluded from their own experimental material that 'lesions of the central nervous system did not occur in vitamin E-deficient rats at any age' *It is of some interest, however,* that changes in the lipid content of the brain have been reported in vitamin E-deficient rats The total lipid and cholesterol concentrations are increased, the free cholesterol portion is said to be elevated (427)

In Summary, vitamin E deficiency leads to disturbances in reproduction irreversable testicular changes and dystrophy of both striated and cardiac muscles

Vitamin E Deficiency in Man Vitamin E has been used in a large number of rather unrelated clinical syndromes without any clear-cut results At this time there is no evidence for the occurrence of vitamin E deficiency in man or any indication for its use in clinical medicine

and Emmel (425) In such animals ceroid (page 195) is found in the myocardial fibers and in macrophages in the interstitial tissues There is evidence of destruction of myocardial fibers but this seems to be a very slow process The most conspicuous change is the presence of a great deal of connective tissue separating the myocardial fibers, ceroid-laden macrophages are found

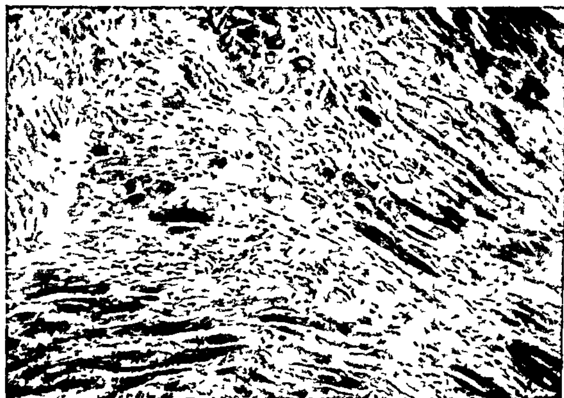


FIGURE 39 Heart Vitamin E Deficiency Myocardium of rat on a vitamin E deficient diet for over a year showing extensive scarring and disappearance of myocardial fibers There is in addition some cellular infiltration many of the cells are macrophages containing ceroid pigment There is no evidence of any fresh necrosis of myocardial fibers ×150 (Courtesy of Dr Karl E Mason)

here This is the sole recorded observation of lesions in the cardiac musculature produced by vitamin E deficiency *Physiological studies indicate that the* deficient rabbit heart is less resistant to posterior pituitary extract but more resistant to such cardiac glucosides as oubain and digitoxin (426) In contrast to changes in skeletal muscle no alterations in the lipoid or cholesterol content of vitamin E-deficient cardiac musculature have been observed (427)

Attention has also been called to lesions of the kidneys in vitamin E deficient rats (773), the nature of the change needs clarification Necrosis of the tubular epithelium has been described this becomes more marked as the deficient state progresses

Mason and Emmel (425 428) have carefully studied a curious pigmentation which a number of investigators have noticed in vitamin E-deficient

ished clotting power of the blood is the complete explanation of the bleeding because it requires the assumption that in ordinary activity, with attendent traumatization the clotting mechanism is constantly being called into action in normal individuals

In the rat hemorrhages occur in numerous sites most commonly in the subcutaneous tissues of the lower extremities Bleeding is also seen in the thymus which may be greatly distended by red cells bladder, eye adrenal, testis kidney retroperitoneal tissues and the various cavities (443) Another interesting study in the rat has described the presence of multiple hemorrhages in the brain and the possibility that the altered physico chemical properties of the blood mainly hypoprothrombinemia which may interfere with the proper hydrodynamics of the blood circulation might affect structurally the blood vessels' (767)

A most interesting observation in pregnant rabbits has been reported by Moore et al (444) who find that animals which are fed a vitamin K deficient diet consistently abort during the early stages of pregnancy (8-14 days after mating) Microscopic examination reveals the presence of fresh and old hemorrhage in the decidual plates of such animals

Vitamin K Deficiency in Man Clinical hypoprothrombinemia with its resulting hemorrhagic manifestations is observed in patients with obstructive jaundice diarrhea sprue et cetera and is too familiar to warrant much consideration here Vitamin K has been very effective therapeutically

The vitamin has also been used to combat the hypoprothrombinemia which is observed in the new-born As numerous investigators have demonstrated there is a rapid fall in the prothrombin content of plasma at birth In addition there is virtually no vitamin K in the new-born infant during the first week of life The placenta is not permeable to vitamin K after delivery the prothrombin derived from maternal sources is soon used up Following a certain period usually about a week vitamin K begins to be elaborated by the intestinal flora the blood prothrombin level therefore rises (445) It is thus apparent that vitamin K therapy will materially affect the prothrombin levels and bleeding tendencies of new-born infants

Data from the Johns Hopkins Hospital (446) indicate that in those mothers who do not receive vitamin K antenatally 4 1 percent stillbirths or neonatal deaths occur while in the mothers receiving 2 methyl-1 4-naphthaquinone the incidence is only 1 5 percent This as well as other reports on the efficacy of vitamin K therapy in reducing infant mortality have been questioned however Potter (447) in the largest series of new-born infants to be reported has presented evidence which indicates that the mortality rate is not altered by the routine administration of vitamin K to women during labor At the present time the efficacy of vitamin K before and during delivery needs to be clarified It seems logical however that vitamin K should

Vitamin K

Historical In 1929 Dam described a hemorrhagic syndrome in chicks which had been placed on a diet virtually free from sterols (434) The bleeding tendency was not prevented by ascorbic acid, the cause was ascribed to "a lack of a factor or factors occurring in cereals" (435) In 1935 the same investigator presented evidence that the factor was a vitamin which he designated vitamin K ("Koagulations-Vitamin) (436) During the next few years several laboratories carried out extensive investigations of the active factors which led to an elucidation of the chemical nature and finally the synthesis of a relatively large group of compounds of which 2 methyl 1 4 naphthaquinone (Menadione) is most active (437)

Biochemical Relationships The function of vitamin K in the animal organism is related to the formation of prothrombin 2-methyl-1 4 naphthaquinone and its related compounds are fat soluble Their absorption is enhanced by bile acids since animals in which biliary fistulae have been produced and humans with obstruction of the biliary tract develop hypoprothrombinemia which may be corrected by feeding bile salts (438) That the liver is the site of prothrombin formation has been shown, for in hepatectomized dogs large amounts of vitamin K fail to affect the plasma prothrombin level (439) More precise data on the role of vitamin K in the mechanism of prothrombin formation awaits further elucidation Quick s (440) separation of prothrombin into two components A and B which are combined through calcium is a step in this direction since evidence already accumulated indicates that in vitamin K deficiency there is inadequate synthesis of the B component

The relationship of vitamin K to the anticoagulant factor of 'sweet clover disease' 3,3-methylenebis (4-hydroxycoumarin) or dicoumarol deserves mention (442) Various compounds having vitamin K activity are effective in counteracting this anticoagulant in rats the lives of animals fed doses of dicoumarol which are ordinarily toxic are prolonged when diets containing vitamin K are employed (442)

Pathological Effects Vitamin K deficiency as evidenced by reduction of blood prothrombin level has been demonstrated in several species (438 443 444)

Aside from the physiological defect associated with severe hemorrhage no other changes have been detected in the tissues of vitamin K deficient animals The possible relationship of prothrombin deficiency to capillary integrity is of great theoretical interest and should be studied further As Wolbach and Bessey (317) have pointed out one wonders 'if the dimin-

A large number of rather miscellaneous effects have been ascribed to ascorbic acid, in the main these have been based on experimental observations in deficient guinea pigs It is difficult to interpret and classify such observations so that they can be linked with the pathological manifestations which will shortly be described However certain experimental findings are important and should be mentioned One of the most interesting functions of ascorbic acid appears to be its relation to the metabolism of the aromatic amino acids phenylalanine and tyrosine Both ascorbic acid-deficient guinea pigs (454) and premature infants reared solely on cow s milk (455) excrete parahydroxyphenyllactic and parahydroxyphenylpyruvic acids in the urine when large amounts of phenylalanine and tyrosine are fed Such hydroxy-acids are ordinarily metabolized and not found in the urine This metabolic defect is eliminated when adequate amounts of ascorbic acid are restored to the diet *In vitro* studies in guinea pigs indicate that the main difficulty of vitamin C deficient tissues is an inability to oxidize the side chain of tyrosine rather than a failure to oxidize the benzene ring or conjugate the phenolic group (456)

Other metabolic defects have been observed in vitamin C deficient animals Decrease in the succinic dehydrogenase activity of heart and skeletal muscle has been reported (457) as well as a rise in blood fibrinogen (458) and a decrease in serum phosphatase activity in infants (512) and guinea pigs (459) but not the adult human however (481)

The outstanding investigations of Friedenwald and his associates (460) have implicated ascorbic acid in yet another important physiological process the secretion of intraocular fluid The vitamin is one of a group of reducing substances which are stored in the stroma of the ciliary body When ascorbic acid is restricted by dietary means the content of this substance in the eye falls rapidly so that after twenty-four to forty-eight hours no reducing substance can be titrated in the aqueous There is a coincident decrease in the secretion of the intraocular fluid Friedenwald postulates that the secretion of the aqueous is dependent on differences in oxidation-reduction potential between the ciliary stroma and its epithelium Ascorbic acid thus acts as a moderator in a redox chain connecting the oxidase activity of the epithelium with the dehydrogenase activity of the stroma" In this way water is transferred across the epithelial-stroma barrier

A relationship may exist between vitamin C and the production of adrenal cortical hormone which would be of interest in view of the extremely large concentrations of ascorbic acid in the adrenal gland When pituitary corticotrophic hormone is injected into rats and guinea pigs there is a decrease in ascorbic acid concentration of the adrenals, for instance, in the former species the concentration may fall from 314 mg per 100 gm fresh tissue to 141 mg six hours after adrenotrophic hormone is injected (461) In guinea

always be administered to the mother antenatally or to the newborn after birth to tide the infant over the first week of life until vitamin K synthesis begins in the intestinal tract

Ascorbic Acid

Historical The familiar observations of Lind during the eighteenth century plainly indicated that citrus fruit juices contain a substance which protects against scurvy Although active preparations were isolated during the early part of this century, it was not until 1932 that King and Waugh (448) announced the chemical nature of vitamin C and demonstrated the biological activity of their product which was soon synthesized, its structure was then determined to be that of an hexose derivative (449, 450)

Biochemical Relationships Ascorbic acid is absorbed from the intestinal tract and widely distributed by the blood stream to the tissues Certain organs have higher concentrations than others, a point which has been brought out by chemical analyses as well as by histochemical methods By the former technique ascorbic acid is found in greatest quantities in the adrenal glands In the guinea pig concentrations average 75 mg per gram The vitamin C content of other representative tissues from the same animal have been reported as follows (in mg per gram) liver, 0 10, brain 0 14, kidney, 0 087, heart, 0 088, skeletal muscle, 0 032, testes 0 18 (451) Studies of the distribution of the vitamin by histochemical methods confirm the chemical analyses and aid in a more precise localization of ascorbic acid in cells and tissues In histochemistry advantage is taken of the fact that ascorbic acid reduces silver nitrate with the deposition of silver at the site where the vitamin was present Fine intracellular granules are prominent in the adrenals, corpus luteum interstitial cells of the testis and in the hypophysis (452)

Ascorbic acid furnishes one of the outstanding examples of the importance of species differences with respect to the effect of vitamin deficiencies Vitamin C deficiency may be produced in the guinea pig, monkey and man, the mouse, rat, rabbit and dog do not need an exogenous source of this substance The action of ascorbic acid may be blocked in the first two species of the latter group for Woolley (453) has shown that a 'scurvy-like condition' may be produced in cotton rats and in mice by feeding an homolog of ascorbic acid, glucoascorbic acid In mice for instance, cutaneous hemorrhages are prominent and bleeding from the gingivae is noteworthy the knees and wrists swell and on section the joints are fiery red" It is unfortunate that histological studies have not been made to determine whether the lesions in the bones are characteristic of the scorbutic state

fibroblasts and the absence of collagen formation certain other differences in the capacity of wounds to heal in scorbutic and normal animals should be mentioned The hemorrhage which occurs as a result of the incision is absorbed much more slowly and may never completely disappear Then too although endothelial cells proliferate capillary loops fail to invade the injured area

These histological observations have been extended by others to obtain data on the ascorbic acid content and tensile strength of healing wounds in

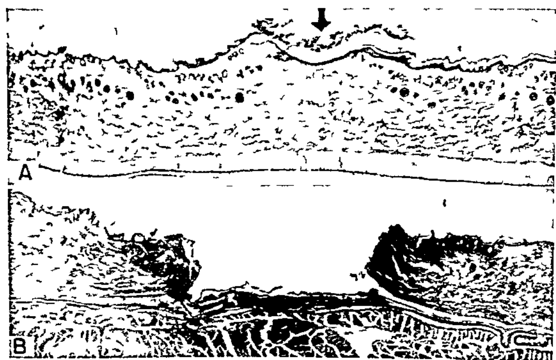


Figure 40 Skin Ascorbic Acid Deficiency *A* Skin from back of normal guinea pig in which an incision had been made nine days before at point marked by arrow Note absence of hair follicles and increased thickness of epithelium to which some debris is adherent The wound is completely healed *B* Skin from a guinea pig suffering from acute scorbutus in which a similar incision had been made nine days before There is no attempt at healing whatsoever and as will be seen a wide defect is present H and E., x6

normal and scorbutic guinea pigs and in humans as well (466 790) The vitamin C content of healing wounds of deficient animals is found as expected to be much lower than that of animals on an adequate intake of this nutrient However there is no increase in concentration of ascorbic acid in the wound site of the deficient guinea pig over the concentrations in the skin elsewhere If air is injected into the peritoneal cavity and the pressure measured until the abdominal wound breaks down the average pressure for wound rupture in scorbutic animals is found to be 127 mm mercury while in controls it is twice as great or about 258 mm mercury In a

pigs the same decreases are noted which indicate the possible relation of ascorbic acid to cortical hormone production

Pathological Effects Numerous experiments have demonstrated that the principal morphological effects of ascorbic acid deficiency are found in mesenchymal tissues In the absence of this vitamin intercellular substances such as collagen osteoid and dentine, fail to be deposited in normal fashion by their respective cells, fibroblasts osteoblasts and odontoblasts There is also said to be a general failure in the deposition of 'intercellular cement substance Rupture of capillaries in particular is a prominent manifestation of the scorbutic state Less specific effects on other soft tissues will be discussed below

Collagen The role of ascorbic acid in the formation of collagen has been studied from several standpoints valuable information having been obtained from investigations of healing sterile wounds, blood clots and subcutaneous abscesses In the former type of experiment the now classical studies of Wolbach and Howe (462) conclusively demonstrate that when incisions are made in the skin of scorbutic animals the lesions fail to heal Microscopic examination of such wounds reveals that although there is extensive fibroblastic proliferation in both scorbutic animals and controls the cells of the former tend to remain immature looking and most important of all fail to deposit collagen in normal fashion From such observations it is concluded that there is failure of collagen formation in the scorbutic state Further studies by Wolbach (463) and others (464 465, 466) have confirmed and amplified the initial observation both in the guinea pig and in man In wounds produced in scorbutic animals a pink-staining fluid like material appears about immature proliferating connective tissue cells This substance has been postulated to be a variety of materials but the feeling of most students of scurvy particularly Wolbach is that this material represents an ineffectual attempt at collagen formation In controlled studies of the recovery processes following absolute scorbutus Wolbach has failed to obtain any evidence that fibrin is a precursor of collagen (463) Instead the homogeneous pinkish-staining material mentioned above which does not take collagen or silver stains becomes fibrillated after treatment with ascorbic acid such fibrillary material is argyrophilic Following the appearance of these reticulum fibers collagen can also be stained Wolbach takes the view that reticulum and collagen are elaborated or secreted by connective tissue cells Particularly important is the observation that following recovery from the scorbutic state collagen deposition is always found in the immediate vicinity of the fibroblastic cells A discussion of the theory which has been brought forward to explain these phenomena will be deferred until the lesions in the bones and teeth have been described In addition to the changes in

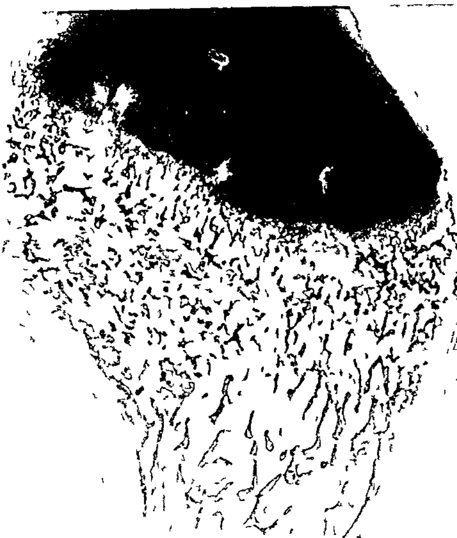


FIGURE 41 Rib Scurvy Costo chondral junction from a six months-old white female infant dying acutely of diarrhea and dehydration Scurvy was diagnosed grossly at autopsy Note widening of cartilage shaft junction and extreme concave deformity of shaft and convexity of the epiphyseal cartilage Spicules of calcified cartilage matrix lie in complete disarray in a marrow composed of immature looking fibroblasts Some evidence of attempts at healing can be made out under higher magnification This illustrates the tremendous destructive effects which the movements of respiration have on this area of structural weakness The cartilage reminds one of a pestle grinding away in the mortar like shaft Note absence of hematopoietic elements in this region H and E x15

on this lime salt matrix and is immediately converted into bone by the deposition of inorganic calcium and phosphorus in it The scorbutic state is characterized by failure of intercellular substances to be elaborated Osteoid, the organic matrix of bone like collagen and dentine is an intercellular substance In scurvy there is a failure of the osteoblasts to form osteoid The entire pathologic picture is thus explained and if this single point is understood the morphologic changes should be clear enough

ing study of six cases of wound healing in the human some correlation has also been detected in the vitamin C concentration of the tissues and their tensile strengths (790)

The histological development of subcutaneous abscesses has been compared in scorbutic and normal guinea pigs, in which the paired-feeding technique was utilized (467) When such animals are innoculated with a strain of hemolytic staphylococcus aureus, microscopically in the first few days there is a prompt outpouring of polymorphonuclear leukocytes in both groups of animals and phagocytosis appears to be active By the third day there tend to be fewer macrophages in and about the lesion of the deficient animals in contrast to large numbers in the controls, there is also little connective tissue proliferation in the former group A week following innoculation the lesion is localized in the deficient animal, but there is a wide zone of connective tissue cells about the necrotic focus, between the cells is a pinkish-staining material and numerous red blood cells are also present No capillaries are found growing into the center of the lesion, instead defective looking' dilated vessels are found at the periphery A week later the zone about the abscess is even wider in the deficient animal in contrast to the compact well-encapsulated lesion of the control From this study it is concluded that although there is no decrease in polymorphonuclear leukocyte response, the macrophage reaction is delayed and is less than that encountered in the control Phagocytosis by the mononuclears also appears abnormal As was expected there is also an inability of the scorbutic animal to produce collagen and organize his abscess Grossly, therefore, the lesion in the deficient animals is diffuse and soft in comparison to that in the controls which is rounded up and firm It is interesting to speculate on the role of blood vessels in relation to the lesion and its pathogenesis Whether defective capillary invasion retards the influx of macrophages is difficult to determine It seems unlikely, however, that poor circulation has any effect on poor collagen formation

Bone Changes in the bones especially those of the guinea pig and growing infant have been reported by a number of investigators since Barlow's (468) description in 1882 (469, 462 470 471) The description to follow is based on a study of experimental scurvy in guinea pigs (472) as well as over one hundred cases of human scurvy which have been observed by the present writer and in collaboration with Dr E A Park and Miss Deborah Jackson

It will be recalled from the discussion of normal osteogenesis on page 106 that growth of the long bones including the ribs takes place by a continuous multiplication and piling up of the cartilage cells which form the epiphyseal plates A continual deposition of lime salts in the matrix substance in the interstices of these cells occurs Osteoid tissue is then deposited



FIGURE 43 Higher power of costo chondral junction of rib shown in preceding figure 42 Note numerous fractures with spicules of calcified cartilaginous matrix material scattered in all directions Although there is an abundance of fibroblast like cells they seem quite impotent of forming collagen or osteoid Changes in the cartilage such as defects in calcification and irregularity in lining up of the cells are due to mechanical factors H and E, ×60

of this zone which determines the resultant pathologic picture It must already be obvious that such spicules of calcified matrix material, unencased in bone and unresistant to the stresses and strains of motion and weight bearing, are especially liable to fracture The changes which accompany such breaks lead to the characteristic lesions of scurvy in the skeleton

The first site of the appearance of fractures is usually at the periphery of the bone where the cortex and the cartilage are in juxtaposition This is probably because there is more displacement here and more pull from attached muscles As the lattice increases in width a more and more fragile zone is developed so that it is inevitable that complete fracture of the spicules of lattice will occur and that separation and various deformities of the cartilage shaft junction will soon follow Such fractures of the calcified



FIGURE 47 Rib Scurvy. Costo-chondral junction from a six months old colored female infant who died of dysentery after an illness of only eight days. The child had never received any orange juice. Scurvy was diagnosed clinically and at the autopsy table. There is widening of the line of ossification with the characteristic concavity of the shaft. Numerous fractures are seen and the region beneath the cartilage is in great disorder. H and E, ×15

As the scorbutic state develops the cartilage cells of the epiphyseal plate continue to proliferate and arrange themselves in rows in normal fashion. So too, lime salts are deposited in the cartilaginous matrix substance between the columns of cartilage cells. However the next step in the orderly sequence of bone growth is deficient or completely lacking—osteoblasts fail to lay down osteoid on the spicules of calcified matrix material. In addition this material is not destroyed. Consequently a wide zone of calcified but unossified matrix develops just beneath the actively growing cartilaginous plate. This formation Park (471) has aptly called the "scorbutic lattice" since it is a "lattice" of calcified cartilaginous matrix material. It is the development

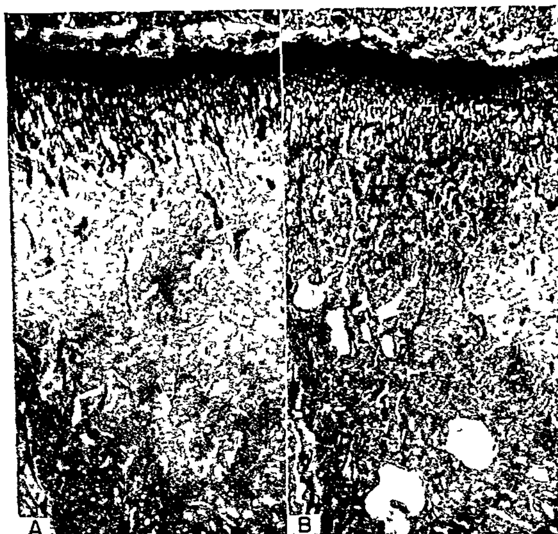


Figure 45 Tibia Experimental Scurvy in the Guinea Pig *A* Tibia from guinea pig completely deprived of vitamin C The picture is similar to that seen in the human save perhaps that the lattice of calcified cartilaginous matrix material is a little broader There are the same fractures and the connective tissue framework with absence of myeloid elements *B* Tibia of opposite leg which had been placed in a plaster cast to immobilize it at the beginning of the experiment Notice prominent lattice which shows no fractures There is no migration of the marrow cells down into the shaft nor is there any evident proliferation of fibroblastic-like cells This illustrates that if the normal stresses and strains of muscle pull are eliminated classical evidence of scurvy with fractures hemorrhages, et cetera will not develop

called Gerustmark' The reason for this migration of marrow cells which leaves only connective tissue elements is not clear

From what has been said it is apparent that the fractures presence of pink-staining material hemorrhage and cellular proliferation are dependent on the development of a structurally inferior zone just beneath the epiphyseal cartilaginous plate That the stresses and strains resulting from muscle pull and motion are responsible for these classical signs of scurvy at the growing

matrix material lead to the classical textbook picture of scurvy, the so called "Trummerfeldzone" or region of complete disintegration. Here beneath the cartilage are found spicules of calcified matrix in considerable disarray lying horizontally and in various other directions. About the fractures and in the clefts there is a pinkish-staining hyaline material. There are large numbers

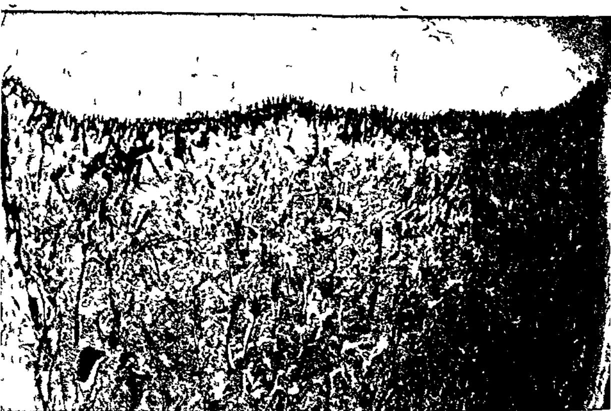


Figure 44. Rib. Healed Scurvy. Costo-chondral junction from a seven months old white male infant who died as a result of diarrhea and dehydration. This is a purely accidental finding at autopsy and was not suspected until the bone was studied microscopically. *There is evidence of old fractures which now are completely healed. The presence of such localized areas of fractures make the diagnosis of healed scurvy a certainty. The line of ossification is perhaps a little irregular but otherwise the bone shows nothing.* H and E., ×15

of red blood cells as well as quantities of apparently impotent osteoblasts, cells resembling fibroblasts but without any vestige of collagen or reticulum in their vicinity. Macrophages containing hemosiderin are also seen. Such is the picture of absolute scorbutus in the guinea pig.

Absolute scurvy in the human is apparently very rare; one therefore usually encounters some evidence of healing. Osteoid and bone are laid down about some of the fractures, the amount of healing varies from case to case, depending no doubt on the degree of the deficiency state. Beneath the Trummerfeldzone is an area where there are no hematopoietic cells and which is composed of a marrow made up of connective tissue cells, the so-

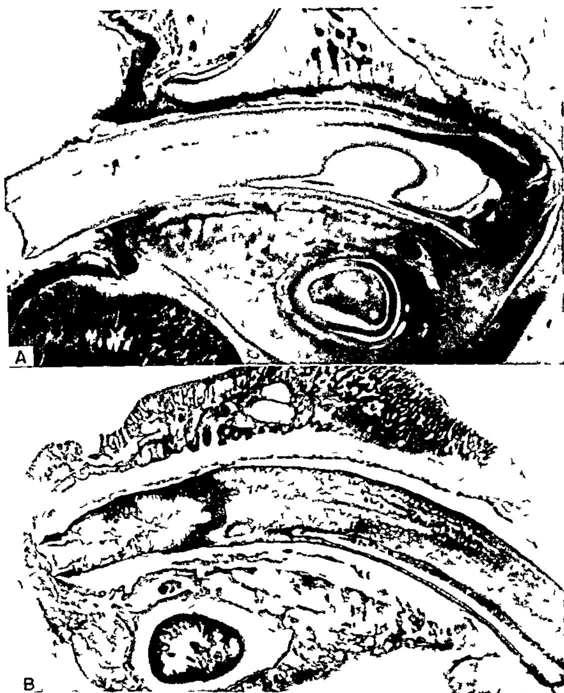


FIGURE 46 *A* Mandible of guinea pig cutting the first molar longitudinally and the lower incisor transversely Acute scurvy There is complete cessation of dentin formation with an atrophic pulp lightly attached to the dentin formed before the deficiency became complete *B* Chronic scurvy This shows imperfect osteodentin formation The peridontal membrane is approximately twice the normal width (Courtesy of Dr Paul E Boyle)

ends of the bone has been shown by the present writer in experiments on guinea pigs (472) One hind leg is immobilized by placing it in a plaster cast, and the animal is then placed on a scorbutic diet When, after a suitable period the two hind legs are examined histologically, the following differences are found At the cartilage shaft junction of the immobilized tibia there is a broad zone of calcified lattice There are no fractures no hemorrhage no pink-staining material no proliferation of fibroblastic or osteoblastic-like cells, nor is there any migration of the marrow cells down into the shaft In contrast the tibia of the opposite side exhibits the classical picture of scurvy with all of the positive findings Such an experiment shows that with the exception of a prominent lattice of calcified matrix material, all of the time honored criteria of scurvy are secondary to the effects of mechanical force on this *locus minoris resistentiae* However, we have been hesitant to make the diagnosis of scurvy in children in the absence of the fractures Gerustmarl and Trummerfeldzone

Certain other features of the skeletal manifestations of scurvy should be mentioned Changes take place in the cartilage of the human but are probably a result of mechanical displacement of the cartilage on the shaft Such alterations consist of a spreading or separation of the cartilage cell columns consequent to the deformities of the costochondral junction Wolbach has commented on unpublished experiments in guinea pigs in which the epiphyseal cartilage becomes defective due to a loss of firmness of the matrix' (317) More detailed information is necessary on this point

Other characteristics of scurvy in the skeleton are rarification of the shaft as a result of resorption This too is a phase of the pathology of the disease which requires further study Subperiosteal hemorrhages develop as a result of trauma and normal stress and strain Macrophages filled with hemosiderin pigment may be prominent following such subperiosteal hemorrhages, those at the cartilage shaft junction have already been mentioned

Teeth and Supporting Structures Histological studies of dental and peridontal structures have been reported in guinea pigs and man Changes in the former species are much more extensive, probably, because the growth of the guinea pig's incisor is so rapid Two millimeters are erupted on an average each week in comparison to only a few millimeters a year in the human (473)

As would be expected the most marked alterations in the teeth are found in the dentine Wolbach and his associates (474 475 476 477) have studied the pathogenesis of the changes extensively When guinea pigs are placed on a scorbutic diet alterations very soon appear in the odontoblasts, these cells become atrophic and soon resemble the nearby pulp cells They loose their orderly polar arrangement and become completely disorganized The vessels of the pulp become dilated and red blood cells ooze through As a re-

studies, he concludes that ascorbic acid is not the decisive factor in maintaining the cohesiveness of sheaths of epithelial cells, and that "the intercellular cement upon which this cohesion depends must therefore be of a different order from the interstitial matrices, which Wolbach and others have found depend on the presence of ascorbic acid for their elaboration." It should further be pointed out that in the bone of a scorbutic guinea pig which was first placed in a plaster cast to eliminate stresses and strains, no pink-staining material appears but is only found where there have been fractures of the calcified cartilaginous matrix lattice (472). Studies of healing in such bones have not been performed and should be carried out in order to determine whether the changes similar to those described by Wolbach in organizing blood clot can be observed. At the present time the "jellation theory" *is an interesting one, but we would prefer to withhold final judgment* and await further evidence.

Certain other tissues have been mentioned as the sites of lesions in ascorbic acid deficiency. Focal necroses of the myocardium have been reported in scorbutic guinea pigs (478). In view of these findings it is of interest to recall that the succinic dehydrogenase activity of heart muscle is decreased in scorbutic guinea pigs (457). It would be of interest to study some of the other enzyme systems in the cardiac musculature of vitamin C deficient animals. When trypan blue is injected subcutaneously into normal and scorbutic guinea pigs, more of the dye is found in the liver and renal tubular cells of the latter animals (480). The findings has been interpreted to indicate a pathological change in the parenchyma of the two organs studied. Anemia does not appear to occur in vitamin C deficient guinea pigs (785).

Ascorbic Acid Deficiency in Man. A discussion of vitamin C deficiency in man can best be divided into scurvy in the infant and young child and scurvy in the adult organism. Criteria upon which to base the clinical *diagnosis of scurvy in children are few, and the disease must be well* advanced before signs are at all significant. Chemical load tests have been employed (482) but have usually not been correlated with the clinical signs and x-ray data. One is therefore at almost a complete loss to estimate the incidence of scurvy in the general childhood population. During the decade 1936-45, 41 cases of clinically manifest scurvy were observed in every 100-000 out patient visits to the Children's Hospital in Boston. This was in contrast to an incidence of 58 cases for the preceding 10 years. During the period 1940-45 an increase occurred, however (781). Data based on morphological criteria furnish more precise information on certain selected portions of the population, that is an hospital population. For instance, when the bones of all children coming to autopsy in the Johns Hopkins Hospital were studied during more than a decade, unmistakable evidence of scurvy was found in 11.7 percent of 487 children between the ages of 3 and 19

sult of the changes in the odontoblasts, dentine is laid down irregularly and the dental tubules are arranged in haphazard fashion. Dentine deposition soon stops entirely. The predentine becomes hypercalcified. A few of the odontoblasts in the pulp apparently form some dentine, at least enough to enclose themselves. The alizarin technique has been employed to demonstrate that dentine formation is quantitatively related to ascorbic acid intake (476).

In the guinea pig changes in the enamel organ come later in the course of the deficiency. The ameloblasts atrophy and hemorrhages are encountered. Both these alterations have been interpreted to be due to traumatic injury of the enamel organ as a result of poor support. There is no evidence of any relationship between ascorbic acid deficiency and dental caries. There is rarification of the alveolar bone, as might be expected when one recalls the changes encountered in the ribs and other bones of experimental animals and humans. Weakness of the supporting bones as well as weakness of the collagen fiber supporting apparatus allows for great mobility and decreased ability to withstand the mechanical stresses encountered in chewing. The changes in the supporting structures of the guinea pig have been likened to the diffuse alveolar bone atrophy of pyorrhea encountered in the human (475).

There is a good deal of controversy among students of scurvy on the mechanism of collagen and bone formation. Wolbach (463) favors the view that collagen material originates as an amorphous ground substance secreted by fibroblasts. He feels that he has shown this in the organization of blood clot in animals which are recovering from absolute scurvy. Then too, evidence is presented that the pinkish-staining material which is found in the Gerustmark of scorbutic bone has "as its basis a product of the cells of the Gerustmark, probably liquid added to by other materials from the blood plasma or cartilage matrix resorption." Such is the basis of the "jellation theory," which postulates that the scorbutic cells deposit a pinkish staining fluid material, which in the absence of vitamin C fails to jell or in other words fails to become osteoid or collagen. The only pointed studies which have been performed are those of Wolbach who adheres to this concept or theory "that the failure of cells to produce intercellular substances in scorbutus is due to the absence of an agent common to all supporting tissues which is responsible for setting or jellying of a liquid product."

Though not contradicting the conclusions of Wolbach with respect to collagen, dentine and osteoid, Chambers (770) has taken issue on the question of intercellular cement substances. As noted elsewhere (page 25) Chambers feels that calcium is an important factor in the formation of intercellular cement which binds epithelial cells together. From tissue culture

gums appeared At the present time based on experimental studies and other observations the feeling is that, few if any cases of gingivitis and bleeding gums result from ascorbic acid deficiency when oral hygiene is maintained In view of the changes of the guinea pig teeth (475), referred to above it is of interest that one of the manifestations which appeared in the human experiment was interruptions in the lamina dura, which "presumably results from atrophy of alveolar bones and is replaced with collagen-free fibrous tissue" (465)

There has been much written concerning the anemia of human vitamin C deficiency The consensus of opinion appears that Vitamin C deficiency anemia does not occur *per se* and that when a reduction of red blood cells and hemoglobin is found this is due to a deficiency of other nutrients (785)

Thiamine

Historical Modern knowledge of thiamine began with the classical studies of Eijkman who demonstrated in 1897 the nutritive value of rice polishings in pigeons fed a diet of polished rice (485) In 1911, Funk isolated from rice polishings a crystalline fraction with biological activity (486) During the following quarter of a century, similar extracts of increasing potency were prepared and used in the treatment of beriberi In 1936 Williams and his associates were able to announce the structural formula and synthesis of a pure and biologically active substance (487) which since it contained sulfur was called thiamine

During the years preceding the synthesis of thiamine, a coenzyme, cocarboxylase which was isolated from yeast, had been extensively studied In 1937 when Lohmann and Schuster showed that this material was the pyrophosphoric ester of thiamine the biological role of thiamine became apparent (488) Thiamine pyrophosphate (cocarboxylase) or diphosphothiamine is made up of a pyrimidine and a thiazole ring plus phosphoric acid

Biochemical Relationships Ingested thiamine is mainly phosphorylated by the liver and to a lesser extent by the kidney Very little free thiamine occurs in normal tissue, the major portion is found as thiamine pyrophosphate (489) The organism excretes thiamine in phosphorylated form *In vitro* studies have demonstrated dephosphorylation by liver kidney muscle and brain tissue (490)

In 1936 Peters called attention to "the biochemical lesion' of thiamine deficiency by showing that when the vitamin was added to suspensions of brain tissue from deficient pigeons the pyruvate content of the mixture was

months (385) The majority of such cases was not diagnosed clinically or, if skeletal lesions were noted, the lesions were usually called rickets Similar studies in other localities are much to be desired The pathology of the osseous changes in scurvy in infants has been detailed above Although a variety of other manifestations have been described, evidence for the specificity of such lesions is not at all clear In view of the morphological and physiological changes in the myocardium referred to above, it is of interest that sudden death has been noted in three infants, 7½, 10, and 11 months of age respectively, all of whom had advanced scurvy of the skeletal tissues two of these children had right sided cardiac hypertrophy at autopsy and no other changes were found to account for death (479) As was noted in a preceding section, changes similar to those encountered in guinea pigs have not been observed in the teeth of the human The tooth germ of two infants aged 8 and 11 months respectively, whose bones showed the characteristic changes of scurvy have been carefully studied but no alterations were found in the ameloblasts or odontoblasts and their intercellular substances enamel and dentine (473) In the younger infant hemorrhages and cysts were observed in the enamel organ No such changes were encountered in the older child Growth of the human tooth is undoubtedly too slow for any dental changes to manifest themselves

Scurvy in adults has of course been described for several hundred years Pathological studies however, have not been carried out on many cases coming to autopsy Following the last war Aschoff (484) described a series of scorbutics pointing out the subperiosteal hemorrhages changes at the cartilage shaft junction and suggestive cardiac involvement Much more information on the pathogenesis of scurvy in the human has come from studies of experimental scurvy of which there have been several reports (465, 481) Crandon s experiment upon himself furnishes extremely interesting data on the biochemical and pathological changes which occur as the scorbutic state develops The plasma ascorbic acid level fell to zero after 41 days on the diet The white cell-platelet ascorbic acid concentration fell to zero after about the 12th week of the deficiency No other significant findings were present until 134 days had elapsed At this time small perifollicular hyperkeratotic papules began to appear Such lesions resembled those previously described as characteristic of vitamin A deficiency (323) After three months a wound which was made in the skin and subcutaneous tissues was found to heal in normal fashion At the end of 182 days after the white cell-platelet ascorbic acid had been at zero for 61 days histological examination of a second wound showed virtually no healing and gave evidence that scurvy based on morphological grounds was present Few other specific changes were found Since so much has been written of the role of ascorbic acid in the etiology of gingivitis in the human it is of interest that no lesions of the



FIGURE 47 Heart Thiamine Deficiency (506) *A* Auricular myocardium of a pig which died suddenly after having been on a thiamine deficient diet for 37 days Note diffuse infiltration with leukocytes many of which are mononuclears There is necrosis of some of the myocardial fibers H and E x150 *B* Lower power (x50) of ventricular myocardium of a swine dying after 156 days on the experimental diet Three episodes of anorexia vomiting and loss of weight had occurred accompanied by a rise in the pyruvic acid level in the blood The first two episodes were ameliorated by the administration of small amounts of thiamine Finally on the day of death the animal became dyspneic cyanosis developed followed by death The section shows large areas where myocardial fibers were necrotic and had been replaced by leukocytes so that a sort of granulomatous lesion resulted Some of these areas could be seen grossly H and E x50

dissociation complete block and auricular fibrillation The tachycardia which has sometimes been observed in experimental animals has been interpreted to be an expression of cardiac decompensation

Objective evidence of cardiac failure has appeared most prominently in swine (506) especially those animals subjected to severe, acute thiamine deficiency Such pigs exhibit labored breathing and cyanosis, both of which are made worse by exercise A number of animals have died suddenly, and no other cause for death save heart failure has been found after careful histological examination of the myocardium and other tissues

reduced (491) Since these now classical studies, the relationship of thiamine to carbohydrate metabolism has been greatly broadened, and it now appears to participate in all oxidative decarboxylations which lead to the formation of CO Thiamine participates in a series of reactions decarboxylation (488), oxidation (53), dismutation (492) and condensation (493)

Pathological Effects Experimental thiamine deficiency leads to disturbances in rats (96, 494), mice (772), hamsters (774, 775), cotton rats (614), cats (495), dogs (496), foxes (497), swine (498), and monkeys (499) Purified diets have not been used in all of these experiments however Poor food consumption is partially responsible for any growth disturbance because of the anorexia and vomiting which usually accompany the thiamine deficient state

Inasmuch as tissue concentrations of thiamine are reduced when this vitamin is restricted from the diet, it is not surprising that metabolic dis turbances may be observed in experimental animals Elevations of blood pyruvic acid have been found in most species For instance, blood pyruvate levels as high as 9 9 mg percent have been observed in monkeys, the average normal value is 3 2 percent (499) Accompanying such alterations in tissue metabolites, a derangement of respiration has been observed in muscle, both cardiac and skeletal brain, kidney, and liver Of particular interest have been *in vitro* studies of the QO_2 of heart muscle (500) in view of the histological changes shortly to be described Although the oxygen consumption of the ventricles of thiamine-deficient and normal rats is about the same, that of the auricles is significantly lower in the former group, the ratio of the oxygen consumption of auricle to ventricle is 1 4 for thiamine-deficient animals and 2 0 for normal controls

Heart In those species in which detailed physiological or pathological observations have been carried out fairly consistent changes have been found in the heart Some time ago bradycardia was described as a distinctive feature of vitamin B deficiency in the rat (501) This observation has been confirmed by other investigators in rats (502 503) dogs (496), swine (504) cats (505) and monkeys (499) The possibility that inanition may lead to bradycardia should be and has been considered In swine studied by the present writer in association with Wintrobe et al (504) thiamine deficiency appears to cause a greater degree of slowing of the heart rate than can be ascribed to inanition alone That vagal overactivity may be a cause of the bradycardia is suggested by slowing of the heart in one animal to which atropine was administered Further evidence of damage to the myocardium has been furnished by electro-cardiographic studies which have been reported *in rats dogs cats* swine, and monkeys Extensive alterations have been described in swine (504) Such changes consist of prolonged P-R intervals abnormalities in the P wave increase of T_4, nodal and ventricular premature beats A-V

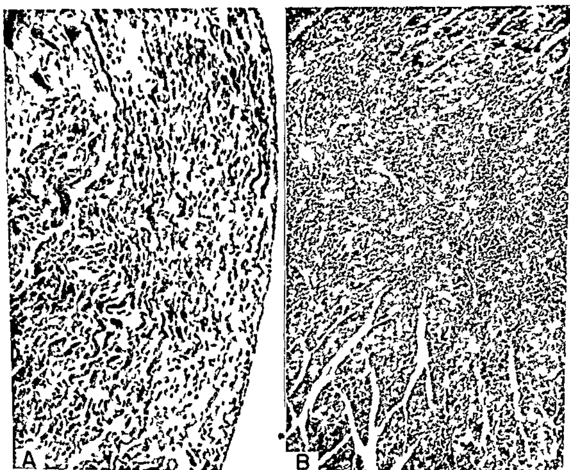


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Inasmuch as tissue concentrations of thiamine are reduced when this vitamin is restricted from the diet, it is not surprising that metabolic disturbances may be observed in experimental animals Elevations of blood pyruvic acid have been found in most species For instance, blood pyruvate levels as high as 9 9 mg percent have been observed in monkeys, the average normal value is 3 2 percent (499) Accompanying such alterations in tissue metabolites a derangement of respiration has been observed in muscle, both cardiac and skeletal, brain, kidney, and liver Of particular interest have been *in vitro* studies of the QO_2 of heart muscle (500) in view of the histological changes shortly to be described Although the oxygen consumption of the ventricles of thiamine-deficient and normal rats is about the same that of the auricles is significantly lower in the former group, the ratio of the oxygen consumption of auricle to ventricle is 1 4 for thiamine-deficient animals and 2 0 for normal controls

Heart In those species in which detailed physiological or pathological observations have been carried out fairly consistent changes have been found in the heart Some time ago bradycardia was described as a distinctive feature of vitamin B deficiency in the rat (501) This observation has been confirmed by other investigators in rats (502, 503), dogs (496), swine (504) cats (505), and monkeys (499) The possibility that inanition may lead to bradycardia should be and has been considered In swine studied by the present writer in association with Wintrobe et al (504) thiamine deficiency appears to cause a greater degree of slowing of the heart rate than can be ascribed to inanition alone That vagal overactivity may be a cause of the bradycardia is suggested by slowing of the heart in one animal to which atropine was administered Further evidence of damage to the myocardium has been furnished by electro-cardiographic studies which have been reported in rats dogs cats swine, and monkeys Extensive alterations have been described in swine (504) Such changes consist of prolonged P-R intervals abnormalities in the P wave increase of T_4, nodal and ventricular premature beats A-V



FIGURE 48 Heart Chronic Thiamine Deficiency (506) Ventricular myocardium of swine which had been on the deficient diet for 246 days There had been numerous episodes of acute thiamine deficiency as evidenced by bouts of vomiting and anorexia accompanied by elevation of the blood pyruvate levels The animal was in a state of severe deprivation for the last 100 days of life with continual elevated pyruvate concentrations In addition to fresh lesions which were found in the auricles and ventricles scars such as those shown in the photomicrograph above were found in areas where muscle fibers had disappeared Such lesions are interpreted to be the sites of previous necroses which had healed Mallory s stain x150

It is of interest to recall that lesions identical with those produced by thiamine deficiency have been observed in animals deficient in potassium (87) When these two deficiencies are simultaneously produced no lesions whatsoever appear in the myocardium (96) The reason for this requires further investigation

No anatomical lesions have been observed in the striated muscles of thiamine deficient animals, however, necroses have been produced in rats in which there is a concurrent potassium and thiamine deficiency (96)

Nervous System Thiamine deficiency has been indicted as the cause of lesions in the nervous tissues Here the pathological alterations are not as clear-cut as are the changes which have just been described in the heart The present state of affairs is perhaps best exemplified by the following two quotations

When the literature of the last 10 years is considered in perspective, the conclusion is inescapable that thiamine has never deserved the title of antineuritic vitamin ' and has not yet shown itself capable of filling completely the role that was formerly assigned to the hypothetical antiberiberi vitamin Meiklejohn 1940 (509)

At autopsy the heart of the thiamine-deficient animal is usually described as dilated (rats, dogs, swine). Evidence for cardiac hypertrophy is extremely difficult to evaluate. Heart weight-body weight ratios which appear to be definitely above normal have been reported in a few of the swine we have observed; it must be emphasized however, that in other animals whose growth is greatly retarded by various means the heart body weight ratio is also found to be increased; the heart may approximate 1.0 percent of the total weight, which is the highest ratio which has been observed in thiamine-deficient swine.

Microscopic lesions in the myocardium have been described in rats (494), dogs (496), foxes (497), and swine (506). The most extensive alterations are found in the heart of the latter species where changes may appear as early as the 37th day of the deficiency. The lesions consist of necrosis of muscle fibers. Initially there is a loss of striations accompanied by vacuolation and hyalinization of the myofibrils. Leukocytes, both polymorphonuclear and mononuclear, then appear. The necroses are either focal or diffuse; in one animal which we have observed they could be seen grossly. The necroses are found in both the auricular and ventricular myocardium. An exception has been noted in one pig dying at an early stage; in this animal only the auricular musculature was affected. A significant difference in the response of the auricular and ventricular musculature in thiamine deficiency is further suggested by observations in rats whose auricles are found to be involved far more frequently then the ventricles. In this connection it is interesting to recall the differences in the oxygen consumption of auricular and ventricular muscle from thiamine-deficient rats which were referred to above (500).

In swine which have passed through several episodes of clinical thiamine deficiency scars may be found at autopsy and have been interpreted to indicate foci where previously there had been necrotic myocardial fibers. The coronary vessels of thiamine-deficient animals, as well as the endocardium and epicardium, are not remarkable; no mural thrombi have been observed in swine. A pointed study of the conduction system has not been made.

The cause of the bradycardia, electrocardiographic changes and morphological lesions is obscure. Since the accumulation of certain metabolic products may be responsible, large amounts of pyruvic acid, sodium pyruvate and related substances have been administered to normal and thiamine-deficient rats (507, 508). Under such circumstances only slight changes are observed in the heart rate and electrocardiogram, so that one must conclude that it is unlikely that accumulations of such metabolites are an important factor. However, it should be pointed out that by such means a sustained elevation of blood pyruvic acid has not been produced. We are, therefore, at a loss to explain the changes specifically except to refer them to the defects in metabolism which are known to accompany thiamine deficiency.

dition in that species as well Such rats display an apparent lameness of the fore and hind legs, they walk with these extremities extended and the gait is weak and unsteady Ataxia also may be present, accompanied by cart-wheel or rolling movements, convulsions have been observed The development and cure of this syndrome in rats as a test for vitamin B_1 was first introduced by McCollum and Simmonds in 1918 (515) and has been used by many subsequent investigators (516), most of whom seem to have had little doubt that they were dealing with animals in which morphological changes were present

In the following discussion the neurological aspects of thiamine deficiency will be divided for convenience into a consideration of the peripheral nerves followed by an appraisal of the central nervous system In evaluating the experiments which are cited below two factors must always be borne in mind In many of the experiments autoclaved yeast has been used as a source of the B group since heat of course destroys thiamine Excessive temperatures may also destroy other components of the B complex, for instance pantothenic acid (522) Secondly animals on a thiamine-deficient diet fail to eat so that the effects of inanition must always be rigidly controlled Finally many of the diets have not contained all of the essential nutrients particularly vitamin K and in birds certain amino acids

The studies of birds on rice diets profoundly influenced the pathologic investigations in other species until careful studies were performed in the latter group Examination of the peripheral nerves of rats fed diets containing autoclaved yeast have revealed no significant morphological differences from control animals (517 518, 519 520) The changes which do appear may be ascribed to inanition In such animals classical signs of vitamin B_1 deficiency (referred to above) are observed When cats are placed on a thiamine deficient diet for as long as 116 days no histological changes can be detected in the peripheral nerves (521) In addition more conclusive evidence is furnished by studies of nerve action potentials of such thiamine-deficient and normal cats No differences are found nor is there any disturbance in the regenerative capacity of the peripheral nerves of thiamine-deficient animals (521) In swine the present writer in association with Wintrobe et al has failed to find any evidence that thiamine deficiency leads to morphological changes in the peripheral nervous system, in particular the sensory neuraxis (506 522)

In the three mammalian species just mentioned no evidence of myelin degeneration of the peripheral nerves has been found In contrast data have been presented chiefly by Swank and his collaborators (523 524) which tend to indicate that in pigeons at least lesions occur in the peripheral nerves Heretofore Aves have not been considered in this book Since the observations of Swank are at variance with those encountered in other species

The most constant and striking findings of thiamine deficiency arise from chemical alterations which lead ultimately to degenerative changes in the nervous system. Changes in the posterior spinal ganglion and anterior horn cells have been noted. Degeneration is most severe in the sciatic nerve and its branches but degenerative changes may be found in any of the peripheral nerves' Spies and Butt 1942 (510)

In 1897 Eijkman described a 'beriberi-like disease' in fowl which had been placed on a ration consisting of polished rice (485) 'The beginning of the disease is characterized by an unsteady gait which first of all manifests itself in walking about on the perch as if the animal cannot squeeze its toes around it firmly enough and must exert itself in order not to fall off. The disturbance in motility soon increases in intensity and speed. The fowl no longer has the strength to climb up, because of weakness it holds its limbs spread apart and bent at the knee and ankle joints, when running it frequently collapses or falls over. Finally it remains lying on its side and in its fruitless efforts the developing paralysis of the wing muscles also becomes noticeable. The paralysis of the body musculature rapidly progresses from below upward"

Eijkman later epitomized the pathological changes (511) 'The involvement of the peripheral nerves is the most important feature that post-morten *investigation reveals to date. It involves both the sensory and motor portions* which occur focally in the nerve trunks and produces the picture of non-inflammatory atrophic degeneration such as is observed after transsection of a nerve in the distal portion of the divided fragment. However, definite changes in the spinal cord and spinal cord roots are also not lacking. These show likewise the appearance of degeneration and atrophy

Based on such findings Eijkman referred to the experimental disease as "polyneuritis gallinarum" In none of his papers are experimental protocols presented or photomicrographs reproduced. Eijkman's experiments were repeated by Vedder and Clark (513) who have illustrated degenerative lesions in the peripheral nerves.

It should be clearly understood that the diets employed by Eijkman and Vedder were composed of polished rice. Such diets are obviously inadequate in many respects as McCollum (514) pointed out many years ago, for besides being deficient in minerals, most of the vitamins in particular the fat-soluble group are not present. Polyneuritis gallinarium" as studied by the early workers is not a syndrome due to a single nutrient but clearly one caused by deficiency of several.

From these studies in birds it was concluded that the purified material from rice polishings was the antineuritic vitamin. When a characteristic syndrome which could be prevented by extracts of rice polishing was also observed in deficient rats, the term 'polyneuritis was applied to the con-

studies of the central nervous system have not been carried out in this species, either.

Lesions have been described in the central nervous tissues of rats. Here, hemorrhagic foci, as well as chromotolysis or clumping of the Nissl substance of the nerve cells, have been noted in Deiter's nuclei, the vestibular nuclei, the nuclei of Bechterew, and the nucleus solatarius. In kittens dying of acute thiamine deficiency, chromotolysis and necrosis of neurons have been observed (530). Small hemorrhages are also said to be present in the vestibular nuclei and there is swelling of oligodendrocytes. The course of such animals with respect to weight gain or loss and the absence of control observations make this study of questionable value, however.

Thiamine has a marked effect on a spontaneous paralytic disease of foxes (497). The syndrome which was first reported from the Chastek fur farm in Minnesota is characterized by a rapidly progressing paralysis. At autopsy, bilateral symmetrical degenerative lesions of certain nuclear masses in the paraventricular regions are encountered. It has been concluded that the Chastek paralysis is the pathologic counterpart of Wernicke's hemorrhagic encephalitis in man, a point which will be discussed in more detail shortly. The disease in foxes results from the presence of a factor in raw fish, which appears to be a thiamine destroying enzyme (533). Although when thiamine is administered to affected animals recovery ensues, deficiencies of other essential nutrients may also be present since at autopsy in animals dying with Chastek paralysis, a severe degree of hepatic lipoidosis is also observed.

Again it is necessary to mention certain lesions which have been described in pigeons by Swank et al. (530) and by Alexander and his group (529). The latter fed pigeons a ration of rice, supplemented with riboflavin and vitamins A, C, and D. Since, on this diet which is obviously inadequate in many essential nutrients, the birds develop hemorrhagic vascular lesions in the brain, Alexander has postulated without any justification whatsoever that thiamine possesses "angiodegenerative properties." Swank describes similar vascular lesions in pigeons as well as changes in nerve cells and fibers, particularly those of the vestibular system and the oculomotor group (530). The latter experiments must be questioned for the same reasons that Shaw and Phillips pointed out and which were discussed above (525). Swank has also applied the technique of electro-encephalography to supplement his morphological investigations (531). During the initial stage of the deficiency the amplitude of the brain waves increases, later there is a reduction in frequency with occasional paroxysmal discharges of epileptiform-like character. It would be most interesting to apply this technique to other species deficient only in thiamine.

In summary, it appears that thiamine deficiency may produce lesions in the brains of Mammalia. Such changes consist of degeneration of neurons,

it seems desirable to mention them, inasmuch as they have assumed a good deal of prominence in contemporary nutrition. When young pigeons are forced-fed diets containing very small amounts of thiamine lesions consisting of myelin and axon degeneration of the peripheral nerves are observed. In assuming that food placed in the pigeon's crop is utilized, Swank has been critisized by Shaw and Phillips (525) who feel that inanition may have led to Swank's findings since "the natural tendency of the bird to reduce its caloric intake during the thiamine deficiency could not be overcome by introducing food into the upper part of the digestive tract." The experiments of Shaw and Phillips lend support to the view that chronic thiamine deficiency may play a role in the development of neurological lesions in birds. They are careful to point out, however, that other factors may be important, included among such factors are the amino acids, glycine and arginine, since the chick requirements for these nutrients are different from those of Mammalia (526, 527). In concluding this discussion of the peripheral nervous system there is no good evidence that thiamine deficiency leads to structural or functional lesions of the peripheral nerves of the Mammalia thus far studied. The question in birds requires further investigation. A discussion of the situation in man will be reserved until later.

Changes in the central nervous system of thiamine-deficient animals may be considered from both physiological and anatomical standpoints. It seems agreed that lesions may occur in some Mammalian species. When rats are placed on a diet whose B vitamin supplement is autoclaved yeast, a significant disturbance of vestibular function appears as evidenced by an increased duration of nystagmus following rotation (528). Physiological alterations have also been studied in thiamine deficient cats whose diets contained adequate essential nutrients including pyridoxine and pantothenic acid (495). The course of the feline syndrome can be divided into three stages. The first, which lasts three to four weeks, is marked by increasing anorexia and vomiting; ataxia is sometimes observed at this time. The second stage is manifested by abnormal posture, ataxia, dilatation of the pupils, and the presence of abnormal reflexes such as body righting, vestibulo-ocular and pupillary light reactions. The flexor, knee kick, and hopping responses are all normal. This stage is followed by one in which convulsions are prominent and are followed by death. From the neurological signs which these cats exhibit it has been concluded that the mid-brain is most severely involved, but it is unfortunate that histological studies have not been carried out to confirm or deny such a supposition. In another study in cats (521) changes as severe as those just described have not been encountered, only ataxia and mild vestibular disturbances were observed. In this experiment it was thought that the animals succumbed as a result of cardiac damage. Ptosis, incoordination, and ataxia have been described in monkeys (499) though anatomical

before passing judgment. Inasmuch too as beriberi must be considered a multiple deficiency disease it is possible that a lack of other nutrients may prevent the effects of thiamine deficiency from becoming apparent in the myocardium. This has some experimental basis, for when there is a concomitant deficiency of potassium and thiamine no cardiac lesions are found although a deficiency of either of these nutrients leads to necrosis of the muscle fibers (96).

In several studies of experimental thiamine deficiency in man manifestations of cardiac abnormality have been extremely insignificant. Electrocardiographic changes of minimal nature have been encountered in a few instances of thiamine deficiency in experimental subjects by investigators at the Mayo Clinic (535). No outspoken evidence of cardiac embarrassment has been detected although it is obvious that it would be hazardous to carry thiamine deficiency too far in view of its known effect on the heart of the experimental animal. In the human then aside from the demonstrated effects of thiamine on the function of the myocardium there is little precise information as to the pathological effects which uncomplicated thiamine deficiency has on the heart muscle fibers. A pointed study of this would seem to be in order in an area where beriberi is, and unfortunately may continue to be endemic for instance South China (536). Extremely valuable information could be obtained if the hearts of persons who die of clinical beriberi were weighed and then examined microscopically with care and if concomitant analyses of the thiamine content of blood cells and heart muscle were performed.

Nervous Tissues Knowledge of lesions which occur in uncomplicated thiamine deficiency in man are equally inadequate. Many cases of clinical beriberi exhibit widespread disturbances in neuro-muscular function paresthesias anesthesias disturbed reflexes muscle tenderness and weakness all are common. Lesions have been described in patients dying of beriberi. Such changes consist of degeneration of the peripheral nerves myelin loss is found in the nerve roots and degenerative changes have been noted in the tracts of the spinal cord. It must be remembered, however that such changes may be due to a deficiency of one or more nutrients other than thiamine in view of the absence of degenerative changes in the peripheral nervous system of animals on inadequate thiamine intakes, it would seem wise at this time to be somewhat cautious in the evaluation of the effects of thiamine deficiency on the peripheral nervous system of man.

Although clinical polyneuritis has been described in human experimental thiamine deficiency the data are not too convincing. For instance a purified diet consisting of casein, fat, sugar salt, and vitamin supplements has been employed to study the effects of thiamine deficiency on a series of individuals for as long as eighteen months (537). The thiamine content

particularly those of the vestibular group. The vascular changes are not clear cut and should be re-investigated utilizing animals on purified diets supplied with adequate crystalline vitamins, not autoclaved yeast. Such diets have been employed by the writer and Wintrobe in swine which lesions of the brain have not been observed even though thiamine was the only nutrient restricted from the diet (522).

Thiamine Deficiency in Man. The pathological manifestations of uncomplicated thiamine deficiency in man are not clearly understood. The clinical disease, beriberi, as it is observed in the Orient, may appear in several forms. 1. "Dry" beriberi with symptoms and signs referable to the neuro muscular system (weakness, paresthesias, sensory loss, etc.). 2. "Wet" beriberi in which there is diffuse edema. 3. Cardiac beriberi, usually manifested by cardiac failure, dilatation of the heart, and elevated venous pressure. As one would expect, mixed types of these 3 forms are not uncommon. However, inasmuch as beriberi of the Orient, especially China, results from diets containing inadequate quantities of polished rice alone, it is reasonable to conclude that the clinical syndrome is a manifestation not only of a caloric deficiency but a multiple vitamin and mineral deficiency as well. The nutritional inadequacy of rice has already been noted (514). Some forms of clinical beriberi, however, apparently do have very close relationships to experimental thiamine deficiency in animals. This subject can best be treated by discussing the cardiovascular and neuro-muscular systems in the naturally occurring disease and in experimental thiamine deficiency in man.

Heart. Electrocardiographic alterations are a prominent manifestation of beriberi and such changes revert to normal when thiamine is administered (534). Changes have likewise been observed in experimental thiamine deficiency in man (535). Post mortem examinations of patients dying of clinical beriberi have revealed relatively little in the myocardium. The heart is said to be enlarged (532) though its weight is not usually mentioned. It is therefore difficult to determine from the available reports whether the enlargement is due to simple dilatation or whether there is hypertrophy as well. The cases of Occidental beriberi studied by Weiss (534) showed simple dilatation in some instances while the heart in other cases showed evidence of hypertrophy. If increase in size of the muscle fibers does occur, the reason for this is not clear. In clinical beriberi there is no hypertension, as a matter of fact Weiss (534) found that the arterioles were dilated. Microscopic examination of the hearts of patients dying from beriberi has revealed very little to date. The presence of "hydropic degeneration" together with mild scarring and sometimes fatty infiltration are the only changes which have been described. It must be emphasized, however, that few careful investigations of the microscopic appearance of the myocardium have been performed, so that it is better to await a pointed investigation of the subject

Wernicke's Disease in man Alexander has concluded that the two diseases are identical and further infers that the latter syndrome results from thiamine deficiency (543) It will be recalled that the diet employed by Alexander consisted of rice fortified only with riboflavin, ascorbic acid, and sources of vitamin A and D, a ration grossly deficient in certain elements and vitamins, especially those of the B complex and fat-soluble group particularly vitamin K It is unfortunate, therefore, that thiamine has been indicted as the sole cause of such lesions in man and pigeons Alterations of the brain in Chastek paralysis of foxes have also been called a "counterpart" of Wernicke's Disease in the human (497) Although thiamine cures the manifestations of the fox syndrome, it will be recalled that animals which die provide at autopsy some evidence that deficiencies in other nutrients are present as well

It is, therefore to be deplored that the hypothesis that thiamine deficiency is the cause of Wernicke's Disease has been rather widely accepted without a full evaluation of the experimental data upon which such a supposition is based The relation of uncomplicated thiamine deficiency to this syndrome is not at all clear at the present time It is gratifying that in a report of forty-two cases of Wernicke's Disease which were studied by Riggs and Boles (539) it is suggested that 'nutritional deficiency forms the basic background of Wernicke's Disease", the authors realize, however, that a multiple deficiency rather than a lack of thiamine alone may be responsible One has only to recall the occurrence of liver disease in alcoholics and the relation of this organ to the production of prothrombin, to wonder whether the hemorrhages said to be pathognomic of Wernicke's Disease may in any way be related to vitamin K deficiency (767) It should further be pointed out that the prothrombin time is increased in choline deficient dogs (675)

It is unlikely that uncomplicated thiamine deficiency ever occurs in man except under experimental conditions Thiamine deficiency on the other hand accompanied by deficiencies in other essential nutrients is widespread at least in certain portions of the Orient, for instance, in South China beriberi is the most important nutritional disease (536) That thiamine deficiency is present is revealed by the absence of this vitamin in the urine (540) Confirmed cases of beriberi are rare in the United States Although Weiss found that the incidence of cardiac manifestations of beriberi occurred in one of every 160 medical admissions in Boston other clinicians throughout this country have failed to confirm such a high rate (541) Cases of alleged beriberi are reported in the literature from time to time the majority of which are instances of cardiac hypertrophy with or without myocardial scarring and mural thrombi (542) Evidence of thiamine deficiency is usually based on a poor dietary history in the subject and no other etiological factor

of this diet was gradually reduced to zero Symptoms and signs appeared in four out of nine subjects and consisted of "neuritis" (otherwise unspecified) edema, anorexia and sometimes vomiting Investigators at the Mayo Clinic (538) placed two individuals on a regimen in which there was 1 mg of thiamine per thousand calories This also led to weakness, anorexia, and vomiting In addition evidence of neuro-muscular involvement appeared numbness and tingling of the legs, sensory disturbances tenderness of the calf muscles weakness of the extremities and loss of the achilles and patellar reflexes It is extremely unfortunate that biopsies of nerve and muscle were not performed on these two subjects to confirm or deny the appearance of anatomical changes especially since fifty days of thiamine therapy were required to correct the defects in one case, while the reflexes of the other subject did not respond even after four months of treatment The observations on these two subjects are the sum total of our knowledge concerning the relationship of thiamine to the integrity of the peripheral nervous system in the human Such evidence unaccompanied by any morphologic data is much too inadequate It is further of some significance that the administration of thiamine to patients exhibiting the clinical signs and symptoms of Occidental beriberi appears to result in far more rapid improvement of cardiac manifestations than those referable to the neuro-muscular system (534) So too Hibbes (295) in a study of the neurological manifestations of beriberi in a Japanese prison camp noted no improvement in 12 men to whom 29 mg of thiamine chloride was administered daily for 3 weeks The clinical characteristics of the disease in the group so studied were sensory in nature Administration of all the vitamin B group lead to some improvement however

Another syndrome allegedly related to thiamine deficiency in man is so-called Wernicke's Disease In 1881 Wernicke described three cases of acute hemorrhagiche Polioencephalitis superior" which were characterized clinically by clouding of consciousness ataxia and opthalmophegia In the disease as it is now recognized lesions are present in the gray matter of the brain and are characterized by small foci of nuclear degeneration with varicose changes of the blood vessels Most striking is the precise symmetrical distribution of the lesions which are usually found in the paramedian and paraventricular nuclei of the thalmus and hypothalmus the region of the habenulae and in several of the cranial nerve nuclei Numerous cases of Wernicke's Disease have been reported particularly in alcoholics

From the previous discussion of changes in the pigeons which were reported by Alexander et al (529) it will be recalled that lesions are said to occur in the brain and to consist of symmetrical foci of damage in the gray matter together with hemorrhages and changes in the blood vessels Because of the similarity of these alterations to the pathological manifestations

this decrease is independent of the intake of the vitamin Riboflavin balance is affected by the thiamine content of the diet, since it has been observed that chronic thiamine deficiency leads to a great loss of riboflavin in the urine a loss which is unaccounted for by body tissue breakdown (549)

The relationship of riboflavin to liver metabolism has been brought out in an interesting series of experiments dealing with the hepatic inactivation of an estrogen estradiol (550) When liver slices from animals depleted in riboflavin are incubated with estradiol they fail to inactivate the hormone whereas normal liver slices destroy it In this connection it is of interest that animals receiving large amounts of estrogenic hormone (7) develop atrophy of the epidermis similar to that seen in riboflavin deficiency (552) Livers of animals deficient in pyridoxine pantothenic acid biotin or vitamin A retain their ability to inactivate estradiol while thiamine-deficient animals react in a way similar to those deficient in riboflavin A relationship of riboflavin to lipoid metabolism has been shown for when high-fat diets are fed to riboflavin-deficient rats such animals survive for a shorter time than those on a high-carbohydrate diet and also develop spastic paralysis' of the hind quarters (551)

Pathological Effects Riboflavin has been shown to be an essential nutrient for the mouse (772) rat (551 552) cotton rat (614) hamster (774 775) dog (554) pig (555) and monkey (556) Prominent changes have been described in the skin the eyes and the nervous tissues as well as certain isolated organs

Skin When growing rats are placed on a riboflavin-deficient diet an initial gain is followed in a few weeks by a loss in weight (552) Skin changes develop after six weeks the fur becomes uneven and ragged and is eventually crusted with a dark reddish-brown substance The hair then becomes loose over the venter which results in a partial alopecia Small white dry scales appear along with these changes in the hair The hair is lost from the eyelids the lips are erythematous swollen and denuded of fur

Microscopically there is an atrophy of the epidermis and its appendages In the early stages there is some hyperkeratosis no inflammation is present Most prominent are the changes in the sebaceous glands whose cells become swollen and then atrophic The rudimentary coil glands likewise atrophy During the early stages the hair follicles remain normal in appearance However the hair which is formed is imperfect Later the follicular cells become atrophic Fully developed riboflavin deficiency is characterized by a skin whose sebaceous glands and hair follicles are almost completely atrophied and whose epidermis has decreased in thickness Following therapy with riboflavin the skin changes undergo involution On the tongue of the rat the filiform papillae of the anterior portion exhibit a defective formation of cornified cells (317)

to explain the lesions It would seem that the only certain proof of thiamine deficiency in such hearts is the determination of the actual concentration of thiamine in the muscle fibers themselves Methods are now available by which this vitamin may be assayed in tissues and it is hoped that such procedures will be applied to the myocardium and to other tissues of persons suspected of dying as a result of beriberi or of any undiagnosed heart disease

Riboflavin

Historical The biological importance of certain yellow pigments from various sources became apparent in 1932 when Warburg and Christian (544) announced the isolation of a yellow respiratory enzyme and showed that it could be split into two portions—protein and pigment Shortly thereafter several laboratories reported the isolation of yellow-green fluorescent pigments from a variety of sources Among this group Kuhn and his associates (545) isolated a flavin which had both the biological activity of vitamin B and a close resemblance to Warburg and Christian's enzyme Kuhn then determined the chemical composition and structure of this active substance which he named "lactoflavin" and in 1935 announced its synthesis (546) Lactoflavin or riboflavin the term adopted by the Council on Pharmacy and Chemistry of the American Medical Association is composed of iso-alloxazine and ribose

Biochemical Relationships Riboflavin is phosphorylated in the intestine The ensuing riboflavin-5-phosphate is then used to build a number of flavoprotein enzymes Riboflavin-5-phosphate is the prosthetic group in Warburg and Christian's original yellow enzyme (544) and in cytochrome C reductase In all other known flavoprotein enzymes riboflavin-5-phosphate is united with adenylic acid to form riboflavin-adenine-dinucleotide the prosthetic group of a variety of proteins which form the complete enzymes which function as hydrogen carriers The effect of riboflavin deficiency on the tissue concentrations of several specific flavoproteins has been studied The concentrations of d-amino oxidase are reduced in the liver and kidney of riboflavin-deficient rats (80) while the same is true of xanthine oxidase content in the liver of similarly depleted rats (547) Riboflavin may be demonstrated histochemically for microscopic study (760)

Studies of riboflavin-deficient rats have revealed no note-worthy changes in the various non-protein constituents of the blood (633) A moderate creatinuria has been observed however A direct relationship has been noted between the protein intake and the riboflavin content of rat liver (548) for when dietary protein is reduced the hepatic content of riboflavin falls and

Ocular Apparatus Corneal lesions have been described in the rat, mouse, and dog The changes have been most extensively studied in the first species In 1939, Bessey and Wolbach (557) carefully described a most interesting manifestation of riboflavin deficiency—corneal vascularization During the end of the fourth week of the deficient syndrome they are able to detect an ingrowth of capillaries toward the center of the cornea The vessels at the limbus seem to serve as the source of these sprouting capillaries In the ensuing weeks new vessels extend further and further, eventually almost reaching the center of the cornea The first vessels grow just under the corneal epithelium The advancing border of the invading vessels is made up of a mass of anastomotic channels with "glomerulus-like loops and arrow-headed-like pointed sprouts" As the deficiency continues the capillaries penetrate deeper into the tunica propria, however only in rare instances are vessels found deeper than the junction of the middle and lower (deep) third of the tunica Soon after vascular penetration of the cornea begins, leukocytes appear and continue to infiltrate the tissue Changes in the corneal epithelium are not observed during the early stages of the deficiency Later on, however although the basal cells remain normal in appearance, the superficial cells become separated and vacuoles form between them and the deeper cell layers Decemet's membrane and the endothelial lining of the inner surface of the cornea appear normal The cornea becomes progressively cloudy and in the later stages of the deficiency ulceration occurs

Following treatment with riboflavin the turbidity of the cornea rapidly clears up Vessels are no longer seen although microscopic study reveals that collapsed capillaries can be observed in animals for as long as two months following institution of therapy

The cause of the ingrowth of capillaries is not at all clear Whether this is a manifestation of damage to corneal epithelium and/or tunica propria cannot be decided at present from histological preparations although corneal vascularization commonly accompanies the damage to these structures and of course may be produced experimentally by appropriate measures (597) It is also possible that the capillaries are a means of supplying riboflavin to cells whose ordinary sources are cut off Bessey and Lowry (558) have demonstrated a reduction in the riboflavin content of the deficient rat's cornea Since the concentration of riboflavin and riboflavin adenine dinucleotide are maximal in the lachrymal and meibomian glands (of the ox at least) (559) it is quite possible that the secretions of these structures are the cornea s source of riboflavin When the riboflavin content of such secretions is lowered by diminished dietary intake it is obvious that the cornea will receive less of this important vitamin

Bessey and Lowry (558) have explored the possibility that visible or ultraviolet light may inactivate the riboflavin of the cornea *in vivo*, inas-

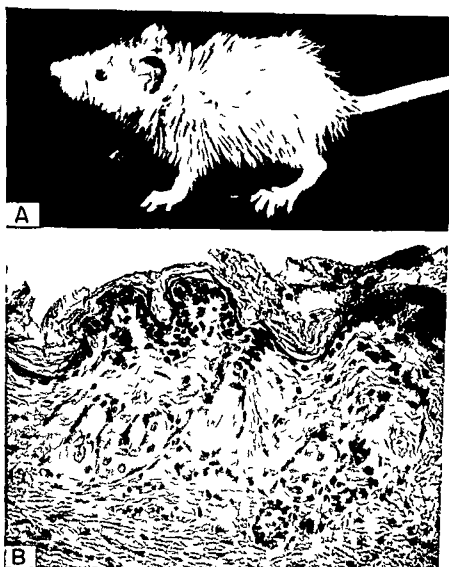


FIGURE 49 Skin Riboflavin Deficiency (552) *A* Rat which had been on a riboflavin deficient diet for about six weeks Note uneven and ragged appearance of the fur and stunting of the animal Alopecia usually begins at this stage *B* Section of skin to show disintegration of sebaceous gland cells with loss of nuclei This change occurs a little later In addition there is beginning atrophy of the epithelium with slight hyperkeratosis (Courtesy of Dr Maurice Sullivan and *The Journal of Investigative Dermatology*)

In the mouse the epidermis microscopically shows either atrophy or hyperkeratosis, there are intra epithelial accumulations of leukocytes (553) The sebaceous glands in contrast to those of the rat appear normal However, *the pathogenesis of the skin lesions in this species is not clear* and further study is necessary Dermatitis" about the mouth has been described in the riboflavin-deficient hamster (774) and when dogs are placed on a riboflavin deficient regimen dry scaling of the skin, accompanied by erythema of the hind legs, chest and abdomen has been observed (554) Erythema and scaling of the epidermis have also been reported in swine (555) and monkeys (556)

lens and consist of "white dot and streak opacities and a few minute vacuoles"

Nervous Tissues Equivocal changes have been noted in the nervous tissues of mice dogs swine and monkeys In the mouse myelin degenera-

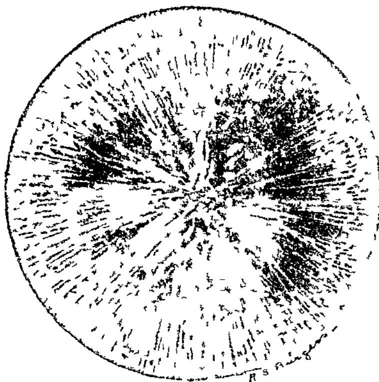


FIGURE 51 Cataract Lens of a riboflavin swine to show opacities (Courtesy of *Bulletin of the Johns Hopkins Hospital*)

tion as evidenced by the Marchi stain has been found in the brachial and sciatic nerves, degeneration in the dorsal columns of the spinal cord has likewise been mentioned (553) In the dog demyelinization of the dorsal columns of the spinal cord and peripheral nerves has been described (563) In one of three swine studied by the present writer myelin degeneration of the sciatic and brachial nerves was observed (555) No histological studies have been carried out in the monkey although when such animals are placed on a riboflavin-deficient diet they develop incoordination a faulty grasp reflex, and loss of strength in the arms and legs (556)

Blood There is some evidence that riboflavin deficiency leads to impairment of red blood cell formation If rats are rendered deficient in riboflavin and then subjected to repeated hemorrhages a marked disturbance of red blood cell and hemoglobin regeneration is found (712) A mild microcytic hypochromic anemia is said to develop in dogs (554 564) while in swine (555) a moderate normocytic anemia develops and in monkeys (556)

much as such radiation leads to inactivation *in vitro* The results, however, have been negative as these investigators have not been able to note any difference in the effects of brilliant and continuous illumination upon the development of anatomical changes in the cornea (560)

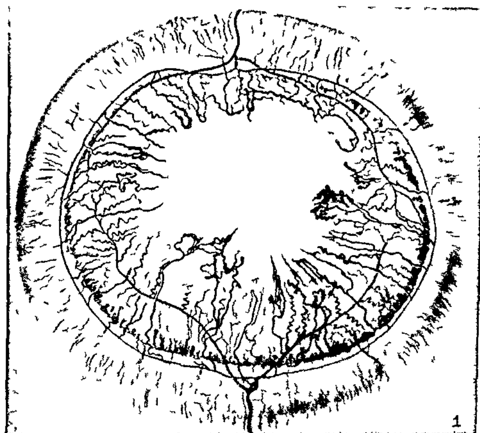


FIGURE 50 Cornea of riboflavin deficient rat There is extensive vascularization of this structure with the ingrowth of many new capillaries (Courtesy of Dr S B Wolbach and the *Journal of Experimental Medicine*)

The lens is another site of damage in the riboflavin deficient animal Cataracts have been observed in rats (561), and swine (555) Day and his co-workers (561) have described lesions in the lens of the riboflavin deficient rat, consisting of a central opacity which spreads peripherally, such cataracts can be arrested by the administration of riboflavin Other investigators have failed to find changes in the lens of riboflavin-deficient animals These discrepancies have been clarified however, by the demonstration that cataracts do not regularly appear when the diet is completely devoid of riboflavin, but make themselves manifest when small but inadequate amounts of riboflavin are administered (562) On a riboflavin-deficient diet 2 of 3 swine developed cataracts after 135 and 145 days of the regimen (555) The cataracts in these animals are located in the superficial portion of the cortex of the



Figure 53 Riboflavin Deficiency and Congenital Malformation Three embryos stained and cleared to show bone lesions *A* Normal control *B* and *C* Newborn rats of riboflavin deficient females Note fusion or non-separation of ribs Note shortening of radius and ulna of *C* as well as absence of tibia and fibula The tibia is not present in *B* The progressive shortening of the mandible in *B* and *C* is also striking (Courtesy of Dr Josef Warkany and *The Milbank Memorial Fund Quarterly*)

Riboflavin Deficiency in Man Lesions which were specifically ascribed to a deficiency of riboflavin have been reported by Sebrell and Butler (586) in a group of women who had been placed on an experimental diet containing thiamine and an unknown amount of the other B vitamins After ingesting such a ration for a variable period changes appeared about the mouth and nasolabial folds In the former site there were macerated areas at the angles of the mouth where fissures formed in addition reddening of the lips along the line of closure and thinning of the mucosa were observed These changes were called cheilosis (a morbid condition of the lips) The nasolabial folds and ala nasi exhibited a scaling greasy dermatitis The cheilosis and skin lesions were cured by the administration of riboflavin In addition to these changes alterations elsewhere were described to be prominent manifestations of riboflavin deficiency in the human Vascularization of the cornea of the experimental animal will be recalled similar changes as well as glossitis were reported in man (569) and led to the widespread belief that riboflavin deficiency was common in this country Subsequent studies have failed to sup-

an anemia has likewise been observed In all of these studies of hematopoiesis the data are too inadequate to permit any general conclusions

Miscellaneous The fat content of the liver is increased in riboflavin-deficient dogs from a normal of about 15 percent to 40 or 50 percent (554) Similarly, of three deficient swine, two have exhibited on microscopic examination rather large quantities of fat in the liver, and in all, the convoluted tubules of the kidney contained globules which could be stained with Scharlach R (556) The possible lipotropic role of riboflavin must be further studied in these two species

For the past several years Warkany and his associates (565, 566) have been studying the effects of maternal nutritional deficiencies on their offspring In rats shortening or absence of the tibia, mandible, fibula, radius, ulna, femur, ribs, fingers, and toes have been observed Fusion of the ribs and cleft palate may also accompany the above changes, all of which have been shown to be prevented by the inclusion of riboflavin in the maternal diet before or on the 13th day of gestation After this critical period abnormalities will appear in the newborn whether or not riboflavin is administered (566) No detailed histological studies of tissues other than the bones of these animals have been recorded It should be pointed out that although the diet first used by Warkany was not a purified one, consisting of cornmeal, wheat gluten sodium chloride, and calcium carbonate, supplemented with crystalline vitamins, conclusive results showing that riboflavin is the protective factor have been obtained on synthetic rations composed of sucrose, casein, fat, salts, and crystalline vitamins

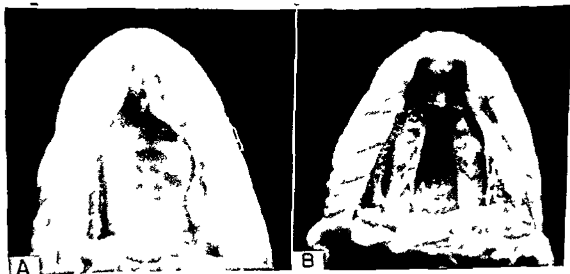


FIGURE 52 Riboflavin Deficiency and Congenital Malformation *A* Normal palate of newborn rat in contrast to *B* cleft palate of animal born to riboflavin deficient mother There is a communication between the nasal cavity nasopharyngeal ducts and mouth (Courtesy of Dr Josef Warkany and *The Milbank Memorial Fund Quarterly*)

Co-enzyme I or cozymase has been demonstrated in certain tissues from dogs liver, muscle and kidney cortex, however, only in the liver is there any significant decrease when nicotinic acid deficiency is present, it appears unlikely, therefore that a failure of tissue respiration as a result of deficiency in cozymase is the direct cause of death in the "blacktongue" syndrome, which will shortly be discussed (579) Co-enzyme I (DPN) is known to be active in the dehydrogenation of hexose monophosphate and triose phosphate, Co enzyme II (TPN) is concerned with the dehydrogenation of lactate, malate, glutamate, beta-hydroxybuturate alcohol and glyceraldehyde diphosphate

Pathological Effects In 1917 Chittenden and Underhill (580) reported a syndrome that had been observed in dogs which were placed on a diet of dried peas cracker meal and cotton-seed oil or lard with or without small amounts of meat This disease was described as follows

"The onset of the pathological symptoms is generally very sudden Usually the first abnormal manifestation is a refusal to eat and examination will reveal nothing to accounting for the loss of appetite The animal lies quietly in its pen and is apathetic After continued refusal to eat for a day or two, the mouth of the dog will present a peculiar and characteristic appearance The inner surface of the cheeks and lips and the edges of the tongue are so covered with pustules as to give the impression of a mass of rotten flesh The odor from these tissues is foul and almost unbearable When stroked with absorbent cotton the mucous lining of the mouth comes away in shreds Intense salivation is present The teeth appear to be solid and normal A bloody diarrhea is present, attempts at defecation being very frequent and resulting in the passage of little more than a bloody fluid of foul odor In some cases the thorax and upper part of the abdomen may contain many pustules half and inch in diameter which are filled with pus organisms No other skin lesions are prominent Death usually results without any particularly striking features

'At autopsy two types of conditions are recognizable In the animals presenting foul mouth and bloody diarrhea the chief interest centers in the lower bowel and rectum which exhibit an intense hemorrhagic appearance With those animals dying rapidly from convulsions the only visible abnormality of the alimentary tract is the presence in the duodenum of one or more large ulcers"

It is concluded that "In the essential features the pathological manifestations described in this investigation closely resemble those which may be observed in human pellagra"

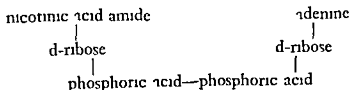
A similar endemic syndrome consisting of anorexia buccal lesions, diarrhea prostration and death was reported in dogs by other observers in this country and received the name blacktongue (581) Goldberger

port such a view, although it is agreed that a few cases of corneal vascularization in the United States do result from riboflavin deficiency The specificity of cheilosis, as well as the changes in the skin and glossitis, has also been questioned (570), since such lesions may be observed in other deficient states However, evidence from such regions as China (571, 572, 573) make it apparent that cheilosis, glossitis and corneal vascularization are certainly related to riboflavin deficiency, since such tissue changes may disappear when only this vitamin is administered However, the possibility that other nutrients play a role must not be overlooked since long term experimental studies of riboflavin deficiency in man have failed to produce any characteristic lesions The controversial question of riboflavin deficiency in man has been reviewed by others (574 575)

Nicotinic Acid

Historical Although nicotinic acid had been prepared synthetically in 1867 and was subsequently demonstrated to occur in many foodstuffs, its importance in nutrition did not become apparent until 1935 In that year nicotinic acid amide (nicotinamide) was shown to be an important constituent of two already well known co-enzymes Warburg (576) demonstrated that the "hydrogen-carrying enzyme of red blood cells" or Co-enzyme II consisted of adenine, pentose, phosphoric acid and nicotinic acid amide Shortly thereafter Euler and his co-workers (577) showed that cozymase or Co-enzyme I likewise contained the amide of nicotinic acid When in 1937 Elvehjem and his group (578) demonstrated that nicotinic acid and its amide were effective in curing blacktongue in dogs these materials came into widespread use in the treatment of pellagra in humans Nicotinic acid is pyridine 3-carboxylic acid

Biochemical Relationships In the organism ingested nicotinic acid is transformed into the amide which is utilized in turn to form Co-enzymes I and II These are heat-stable dialyzable organic substances which function as hydrogen carriers in cellular respiration The chemical nature of these two co enzymes is identical except that Co-enzyme I contains one mol less of phosphoric acid than Co-enzyme II The schematic representation of Co enzyme I, also called cozymase or diphosphopyridine nucleotide (DPN) is as follows



mals never exhibit frequent stools (593) The administration of salt solution prolongs life in some of these animals for as long as 180 days However, though all dogs succumb as a result of nicotinic acid deficiency, the clinical course is somewhat different than that of typical blacktongue in this species Furthermore, Handler has shown that although the blacktongue syndrome can be produced with ease when the classical Goldberger cornmeal-diet is fed, purified rations containing even less nicotinic acid lead to a syndrome which appears either reluctantly or not at all (585) At the same time, others (586) were feeding purified diets containing 19% protein to weanling puppies and observing extensive loss of weight, anorexia inflammation of the gums, and erythema of the palate after 14 to 18 days Similar changes could be produced in adult dogs after 30 to 45 days but, however, were not entirely characteristic of blacktongue in dogs Inasmuch as such deficient animals as well as other dogs on similar diets would not consistently respond to nicotinic acid therapy the ration was supplemented with a "folic acid" concentrate derived from solublized liver extract Following the same procedures employed before it was found that the dogs responded uniformly to nicotinic acid therapy and did not tend to relapse Dogs deficient in nicotinic acid but receiving adequate folic acid" have a lower incidence of buccal lesions, which may indicate that this part of the blacktongue syndrome is not due to nicotinic acid deficiency (588) Inconclusive studies of the blood were reported in these two groups of experiments, this is unfortunate since Handler has shown that dogs on a cornmeal-ration develop a progressive anemia which in some animals is macrocytic in character, the hypothesis that decreased red blood cell formation results from an inadequate supply of cozymase which is needed for the respiration of the immature erythrocyte was proposed (587)

The many inconsistencies in the mode of action of nicotinic acid are gradually being clarified by experiments in dogs (589) rats (590 591) and swine (592) When the former species is placed on a cornmeal-containing diet animals do not gain weight unless fairly large nicotinic acid supplements are administered So too when rats are given a cornmeal ration added nicotinic acid is necessary for good growth Casein supplements do not necessitate the addition of nicotinic acid so too tryptophane has the same effect as added casein When thiamine riboflavin pantothenic acid and choline are furnished together with an optimal amount of dietary protein (26 1 percent) no ill effects can be demonstrated in swine However when the protein content of the nicotinic acid-deficient diet is lowered to 10 percent signs of nutritional deficiency appear Such animals grow poorly their coats are rough and untidy and diarrhea also develops In addition some exhibit a normocytic anemia Chromatolysis of the small dorsal root

(582) had produced pellagra by dietary means in the human and was able, by feeding a similar diet to dogs, to reproduce the black tongue syndrome of Chittenden and Underhill The disease produced by Goldberger could be prevented by meat or yeast In the same month and year that Goldberger's paper appeared Underhill and Mendel (599) published a report dealing with a continuation of previous investigations of blacktongue at Yale Their diet which contains meat and yeast led to typical blacktongue which could be prevented by adequate amounts of cod liver oil or "carotine" It thus appeared that a strikingly similar syndrome could be produced by a deficiency of two different nutrients This situation was further studied by Smith et al (289) who reproduced an identical disease in dogs utilizing both types of diets in order to study the buccal flora of animals manifesting oral lesions, in both groups large numbers of the fuso-spirochetal group of organisms were found The question of these two types of dietary blacktongue if, of course, they are actually different, is certainly a most interesting one and should be re-opened in view of the newer developments shortly to be described

The pathological changes occurring in the tissues of Goldberger's dogs were studied by Denton (583), who found microscopic lesions in the mucous membranes of the mouth, pharynx esophagus, intestines, and scrotum The change was interpreted as a "degenerative process affecting the superficial connective tissue of the mucous and dermal membranes Changes in the supporting tissues of these mucoid membranes are followed by secondary ones in the epithelium The lesions tend to terminate in an extensively necrotic and diphtheritic inflammation of the upper alimentary tract" Denton likened to changes in the dogs similar lesions which he had previously observed in human pellagra (584) and which are described on page 171 From the description of lesions in Underhill and Mendel's experiments (599) it would seem that these, too, were similar to the changes just mentioned

As was noted previously, in 1937 Elvehjem and his group reported that nicotinic acid cured blacktongue in dogs (578), an observation which seemed to lay to rest the etiology of this syndrome and the role of nicotinic acid in nutrition The matter was not so easily settled, however, since several facts led Handler and his associates to question, and properly so, the role of nicotinic acid in the blacktongue syndrome In the first place, no significant differences can be detected between the cozymase content of certain tissues of normal dogs, and animals succumbing with the manifestations of blacktongue (579) Furthermore, animals which are about to die of typical blacktongue can be saved by the parenteral administration of salt solution This effect does not appear to be due to a replacement of fluid lost from the gastro-intestinal tract as a result of diarrhea, since some ani-

if any, physiological abnormalities, moreover no morphological disturbances have been reported even when the daily nicotinic acid intake is as low as 3 mg per person per day (596) From a review of Goldberger's work and that of others the conclusion is inescapable and is consistent with the experimental studies already referred to that the disease pellagra results from a diet whose protein is of poor quality whose nicotinic acid content is low (595) and which may contain either an anti nicotinic substance or a toxic material (769) Therefore, it now seems clear enough that the classical pellagrin with his smooth red tongue diarrhea symmetrical dermatitis and neurological manifestations seldom evidences the effects of a single nutritional deficiency As in the experimental animal the specific effects of a lack of dietary nicotinic acid alone on the human are not at all clear at the present time

Pathologically the disease pellagra, is usually characterized by changes in the skin, the tongue and buccal cavity the esophagus colon and nervous tissues, in addition there may or may not be an anemia of macrocytic or microcytic type

The pathogenesis of the common form of skin lesions which can be ascribed to nicotinic acid deficiency appears to be as follows (584 768) the initial change is found in the superficial portion of the corium where there is rarefaction of the tissue and dilatation of the blood vessels this corresponds to the erythema observed clinically At the same time changes are seen in the epithelium where there is a disturbance in keratinization Hyperkeratosis and parakeratosis are prominent and may be detected in skin which appears grossly normal Acanthosis is found in skin from clinically affected areas The changes in the corium lead to separation of the epidermis from this structure over extensive areas resulting in bullae The sebaceous glands may become atrophic while the sweat glands show no alteration Certain conditions seem to favor the distribution of lesions heat and sunlight (290) as well as vascular stasis scars burns pressure and inflammation (297) All of these factors imply some interference with the normal metabolism of the skin

Somewhat similar changes are found in the mouth and over the tongue esophagus, and vagina where in all these tissues extensive dilatation of the blood vessels with atrophy of the overlying epithelium may be observed There may be complete disappearance of the lining epithelium of the buccal cavity with grayish areas of necrosis, these on section appear as ulcers teeming with organisms

Extensive lesions are found in the colon where the epithelium becomes atrophic and cysts filled with mucous and polymorphonuclear leukocytes are found ulcers then appear It is of interest that the intestinal lesions

ganglion cells has been encountered 4 out of 5 such deficient animals There is no myelin degeneration in the peripheral nerves however In animals on low protein intake, but receiving nicotinic acid, growth is impaired, but anemia diarrhea, and neurological lesions have not been observed It would be most interesting to administer tryptophane to swine which had been placed on such a nicotinic acid deficient low-protein diet

The role of tryptophane in nicotinic acid deficiency has been further and fully elucidated by Perlzweig and his associates (594), who have shown that the administration of tryptophane leads to an increased urinary excretion of methyl-nicotinamide so that it would appear that nicotinic acid may be formed *in vivo* from dietary tryptophane If this be so, a complete re-evaluation of tryptophane and/or nicotinic acid deficiencies in several species is definitely indicated

The fact that tryptophane may be a precursor of nicotinic acid has not completely solved the blacktongue and pellagra problems however The relation of cornmeal-containing diets to these syndromes has always been difficult to interpret ever since Goldberger's classical experiments on the production of pellagra in humans and blacktongue in dogs (282) In addition to the fact that the tryptophane content of cornmeal is low, the participation of this foodstuff in an entirely different manner in the production of blacktongue and possibly pellagra has been recently raised by Woolley (769) who has isolated a "pellagragenic" agent from corn This material which has been characterized as a pyridine base is a substance whose mode of action is not as yet understood Whether it is an antagonist or anti-nicotinic acid substance or whether it is a material which is toxic for the organism are possibilities which remain to be settled The blacktongue and pellagra questions are therefore still not entirely clear Particularly puzzling is the observation already eluded to, that the mere administration of salt solution prolongs life for many days and as will be seen below rest is also beneficial while sunlight is extremely deleterious in human pellagra

Nicotinic Acid Deficiency in Man Ever since 1937, when the signs and symptoms of the pellagra syndrome were shown to be ameliorated by nicotinic acid this substance has been considered by many to be the specific nutrient whose absence is responsible for the characteristic dermatitis glossitis, and gastro-intestinal disturbances and by some the anemia and cerebral manifestations of this disease as well Such a supposition was natural because of the dramatic response of many pellagrins to nicotinic acid therapy In view of the recent developments of our knowledge of nicotinic acid metabolism it is of interest and significant that experimental nicotinic acid deficiency in the human on an otherwise adequate ration has led to few

reproduced in Denton's (584) report are very reminiscent of changes that have been observed in pantothenic acid deficiency in swine (610). We have recently had an opportunity to examine the tissues from a series of pellagrins which came to autopsy at Duke Hospital and were much struck by the similarity of the end stage of the lesions in the human and those which we had observed in swine deficient in pantothenic acid. Alterations in the nervous tissues in pellagra are much less clearly understood. Chromotolysis of ganglion cells in the brain appears to be fairly prominent, however, myelin degeneration has also been reported. The anemia which usually occurs may be macrocytic in character, an observation which points further to the concept that pellagra is a multiple deficiency disease is that "folic acid" has a therapeutic effect on this macrocytic anemia associated with pellagra.

Now that the metabolism and inter-relationships of nicotinic acid to other nutrients are more clearly understood it will be of interest to re-investigate the entire subject of pellagra in the human and to determine if possible what the specific effects of nicotinic acid deficiency may be in this species. Such a study could doubtless be accomplished by furnishing all of the essential nutrients on a corn-free diet except nicotinic acid and tryptophane, the latter being supplied in varying amounts in a fashion similar to that employed in the experimental study of methionine and cystine deficiencies (page 82).

Pantothenic Acid

Historical. In 1933 Williams and his associates (600) announced the isolation of a new growth factor for yeast. Since the factor was an acidic substance which could be demonstrated in a wide variety of living cells, Williams named it 'pantothenic" (derived from the Greek 'from everywhere') acid. During the next few years work went forward on the occurrence, chemistry and biological activity of the new compound so that by 1940 Williams' laboratory was able to announce the synthesis and the structure of biologically active pantothenic acid (601, 692).

From the very beginning of his experiments Williams had expressed the belief that pantothenic acid was a water-soluble vitamin; in fact he had suggested in 1933 that the material might be related to vitamin G (600). Not until 1939, however, was pantothenic acid shown to be an antidermatitis factor for the chick. SubbaRow and Hitchins (603) then demonstrated that this compound was a growth factor for the rat.

Biological Relationships. The function of pantothenic acid in biological

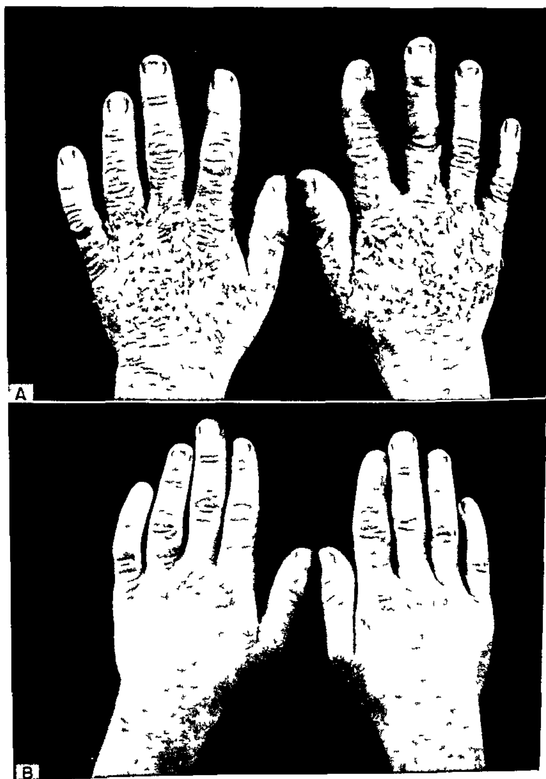


FIGURE 54 Pellagra Dermatitis *A* This 54 year old white male was admitted with an erythematous scaling over the dorsum of the hands diarrhea sore mouth and tongue nausea vomiting and anorexia Diet had been inadequate It was said that the lesions on the hands followed prolonged exposure to a baking oven He was given fluids and a basal diet containing no B vitamins for eight days without improvement On the ninth day ninety mgm of nicotinic acid were administered intravenously There was a steady improvement of the tongue within 24 hrs two weeks later the hands appeared as in *B* and exposure to a heat lamp failed to provoke a relapse (Courtesy of Dr D T Smith (291) and the *Southern Medical Journal*)

Pathological Effects The indispensability of pantothenic acid has been demonstrated for the rat (606) mouse (772) pig (610) hamster (774 775), cotton rat (773) dog (612) and monkey (296) Microscopic studies of tissues have only been performed on the first three species

Skin Specific lesions in the skin and hair have been described in the rat *The pathogenesis of the cutaneous changes have been carefully studied by*

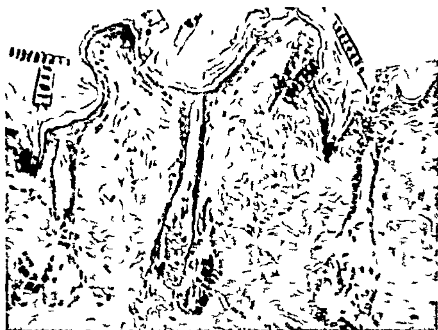


FIGURE 56 Skin Pantothenic Acid Deficiency (606) Section of skin from head of animal which had developed alopecia. There is dilatation of the hair follicles which is characteristic of this deficiency No changes are found in the sebaceous glands corium, or epithelium (Courtesy of Dr Maurice Sullivan and *Archives of Dermatology and Syphilology*)

Sullivan and Nicholls (606) There is first a circumocular loss of hair (spectacle alopecia) The hair is also lost in the preauricular region and sides of the snout This alopecia is sometimes accompanied by scaling Graying of the hair has been observed in piebald rats, being prominent in the circumocular region sides of the nose and shoulders The fur becomes dull and coarse The graying (achromotrichia) is followed by a generalized scaling and erythematous dermatitis Occasionally foci of eczematous dermatitis are also observed Following these epidermal changes the hair begins to fall out

Microscopically there is moderate hyperkeratosis and acanthosis together with an occasional focus of intraepidermal vesiculation and crusting especially where the small eczematous foci had been noted grossly As the rats become more depleted in the vitamin, the epidermis approachs its usual thickness or even becomes atrophic A consistent change is found in the hair

processes is not at all clear Indirect evidence of its possible role in carbohydrate and lipoid metabolism has been advanced After the administration of a solution of 50% glucose to rabbits, the expected hyperglycemia occurs, however, this is accompanied by a 20-30% reduction in the blood pantothenic acid concentration (604) When the blood lipids of pantothenic acid deficient dogs are studied a decrease in blood cholesterol cholesterol



FIGURE 53 Fur Pantothenic Acid Deficiency (606) Head of rat which had been on a pantothenic acid deficient die for about five weeks Note symmetric graying (achromotrichia) of hair about eyes ears and nose This usually spreads to involve the entire head later alopecia occurs (Courtesy of Dr Maurice Sullivan and the *Archives of Dermatology and Syphilology*)

esters, lipoid phosphorus and total lipids are found (605) such data may be significant particularly in relation to the fatty livers which have been observed in some species (see below) It must be borne in mind, however, that similar alterations may result from inanition or the absence of other unknown dietary factors



cytes In addition cystic glands bordered by flat epithelium and filled with polymorphonuclear leukocytes can be observed These lesions may go on to complete ulceration with loss of the entire mucosa All H and E *A* and *B* ×125 C ×150 (Courtesy of *Bulletin of the Johns Hopkins Hospital*)

follicles whose lumens become dilated from orifice to bulb The hair is lost at this time The changes in the sebaceous glands are usually insignificant until the terminal stages of the deficiency when these structures undergo atrophy Little cellular infiltration is found in the corium at any time

Achromotrichia which has been observed in pantothenic acid deficient rats has been corroborated and clarified by Henderson et al (138) These investigators showed that pantothenic acid is not the only chromotrichia factor Copper deficiency (page 52), also leads to graying of the hair and evidence for the existence of a third factor para aminobenzoic acid has also been presented (page 206)

In the skin of mice hyperkeratosis, followed by atrophy of the epidermis has been described (609) There is no inflammatory reaction No mention is made of the conditon of the hair follicles or sebaceous glands Alopecia has been reported by others (772) A "red incrustation" has been described about the mouth of hamsters (774), while the cotton rat exhibits an unspecified dermatitis (612) Alopecia occurs in swine and on microscopic section there is atrophy of the epidermis and loss of hair follicles (610)

Intestinal Tract Diarrhea is an early and constant symptom of the pantothenic acid-deficient swine (610) The stools frequently contain mucus and sometimes blood Rectosigmoidoscopic examination reveals a diffusely hyperemic mucosa which is slightly edematous Bleeding usually occurs as a result of instrumentation Ulceration has not been detected by this method of examination, however

At autopsy extensive changes are found in the intestine particularly the colon Grossly the earliest change is a diffuse hyperemia which has appeared after four weeks of the deficient regimen The lymphoid follicles are enlarged and on section contain purulent centers Perforation of these abscesses leads to small ulcers which become confluent The mesenteric lymph nodes are enlarged Microscopically there is a change from the normal glandular mucosa made up of large vacuolated cells to a mucosa composed of glands lined by atrophic cells Leukocytes are found in the lumina of these glands, as well as in the interstitial tissues about them Although this alteration is a focal one in the early stages it becomes more and more diffuse as time goes on The glands become dilated with accumulations of cells and the atrophic epithelium becomes more and more flattened In the lymphoid follicles which also contain glandular prolongations in their centers the same lesions are found Here following necrosis of the epithelum abscesses develop, these finally rupture leaving large ulcers Following treat-

→

FIGURE 57 Intestine Pantothenic Acid Deficiency (610) *A* Normal epithelium of colon Note cells filled with mucus lining the glands There is relatively little cellular infiltration in the interstitial tissues *B* Early changes in pantothenic acid deficiency Note that the cells have become atrophic and have lost their mucus vacuoles In addition an increased number of cells have appeared in the interstitial tissues *C* This shows a more extensive change with beginning ulceration of the superficial portion of the mucosa in which there are many leuko-

ment with calcium pantothenate the ulcers heal and the intestinal wall at autopsy is found to be thickened due to an increased amount of connective tissue apparently a result of the tissue destruction and healing of the previous inflammation. Changes have not been described in other species.

Harderian Gland. Another manifestation of pantothenic acid deficiency in rats was first described as "blood caked whiskers." The nose and hairs of the snout become covered with a reddish pigment. The source of this material, which is said to be corproporphyrin, has been shown to be the Harderian glands. The assumption has been made that the pigment is excreted through the nasolacrimal duct in pantothenic acid-deficient animals, for when the Harderian glands are excised and the animals are then placed on a panthothenic acid deficient regimen the chromodacryorrhea fails to appear (607). Dehydration may also be a factor (615) leading to pigment incrustation of the nose and whiskers of rats.

Nervous Tissues. During life pantothenic acid-deficient swine evidence nervous tissue involvement by a disturbance in gait. The initial evidence of this phenomenon is a sudden elevation of one of the limbs from the ground as though it were painful. The gait exhibits a broadening base and a jerky "goose step" appears. As the deficiency progresses the gait is more and more impaired so that finally the animal is unable to walk at all and lies prostrate.

Microscopic examination (611) reveals that the earliest change is chromotolysis of the dorsal root ganglion cells. These alterations have been observed in animals in which ataxia had not been detected during life. The ganglion cells exhibit the classical signs of disintegration and lysis of the Nissl substance; cells of all sizes seem equally involved. When the spinal or peripheral nerves of such animals are examined by appropriate techniques, no changes are found in the early stages. However, later, that is from the eighth week of the deficiency on, loss of myelin and axis cylinder degeneration are found in the brachial and sciatic nerves. As time goes on changes may likewise be observed in the dorsal root fibers and in one animal degeneration of some of the fibers in the dorsal columns has been noted. Chromatolytic cells have been found in the anterior horns and intermediate gray matter of a small number of animals. Using the osmic acid technique, which is notoriously unreliable, myelin degeneration has been reported to be present in the dorsal columns and pyramidal tracts of the spinal cord and in the peripheral nerves of pantothenic acid deficient mice (609).

Adrenal Gland. One of the most interesting manifestations of pantothenic acid deficiency is the appearance of so-called "hemorrhagic necrosis" of the adrenal glands of rats. The microscopic anatomy has been described by Ashburn (608) but the pathogenesis of the adrenal changes is not at all clear and should be studied more carefully. In deficient rats the following

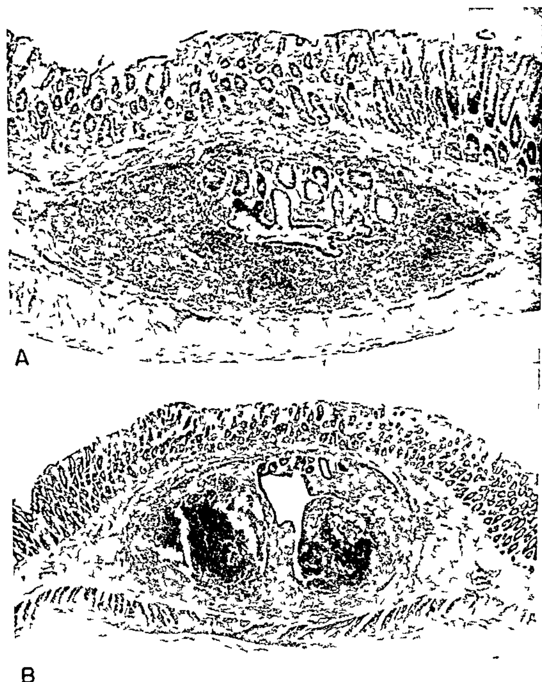


FIGURE 58 Intestine Pantothenic Acid Deficiency (610) Lesions in the solitary follicles of the colon *A* Solitary lymphoid nodule from pantothenic acid deficient pig showing the normal prolongation of glandular elements into this structure There is beginning leukocytic infiltration of the glands The lymphoid tissue is somewhat hyperplastic *B* More advanced lesion which has become two abscesses in the middle of the follicle Such abscesses grow and suppurate producing ulcers Both H and E ×75 (Courtesy of *Bulletin of the Johns Hopkins Hospital*)

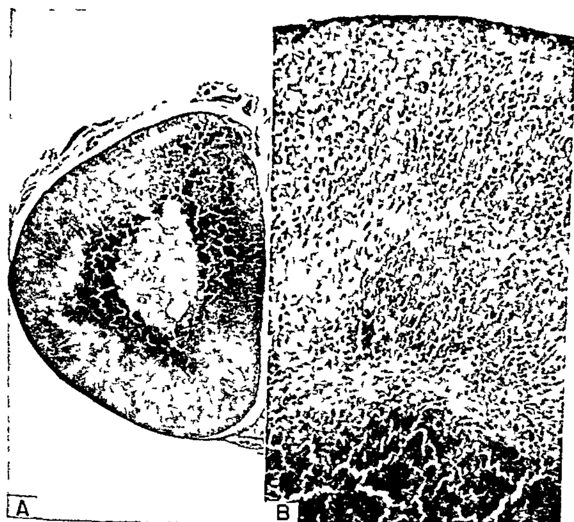


FIGURE 60 Adrenal Pantothenic Acid Deficiency *A* Low power (x55) of adrenal from pantothenic acid deficient rat Note that the medulla and outer cortex appear normal *B* Higher power (x150) of the same adrenal Note that the glomerular and outer fasicular zones are normal in appearance The inner portion of the latter as well as the outer reticular zone show a focus of fresh necrosis in which there are leukocytes and red blood cells The inner portion of the reticular zone is not affected (Courtesy of Dr Maurice Sullivan)

tailed studies of the blood vessels have been reported The changes tend to be localized to the reticular zone, though the inner portion of the fasicular zone may be involved as well The medullary portion of the gland is said to remain normal A constant finding is depletion of the lipoid content of the cortical cells this is probably a consequence of the accompanying inanition however

Changes in the adrenals have not been found in other species The only possible evidence of any alteration in the adrenal glands besides the rat is a dramatic syndrome in dogs described by Schaefer et al (612) After a variable period depending on the vitamin intake, there appear sudden prostration or coma tachypnea and tachycardia convulsive movements of

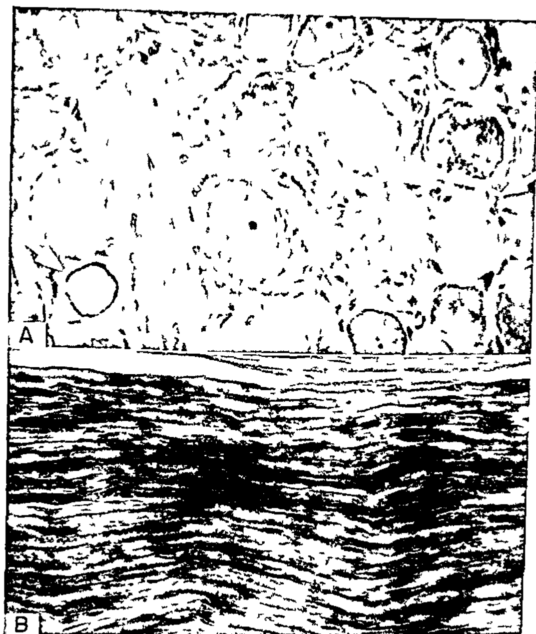


FIGURE 59 *Sensory Neuron Pantothenic Acid Deficiency (611) A Dorsal root ganglion* from lumbar region of pig which had been on a pantothenic acid deficient diet for 37 days. Although ataxia had never been *definite suspicious changes had been present for a* week before death. Note chromotolysis of two ganglion cells (arrows) which is characterized by loss of Nissl substance in one and a condensation of the chromophilic material about the periphery of the cell in the second instance. *B* Sciatic nerve from the same animal. There is no myelin degeneration. *A*, methylene blue. *B*, modified Weigert stain. Both x500 reduced 2/5. (Courtesy of the *Journal of Experimental Medicine*.)

abnormalities in order of frequency have been noted: hemosiderin deposition, fibrosis, "congestion", hemorrhage, cellular atrophy, necrosis and scarring. The constant appearance of hemosiderin in the glands of animals allowed to succumb and its presence in deficient animals treated with pantothenic acid make it highly probable that the initial lesion is a vascular one. No de-

skin lesions sooner and die in a shorter time than animals whose pyridoxine-deficient diets contain lower amounts (15%) of protein A reduction in the urinary excretion of creatine and uric acid and elevation of these substances in the blood have been reported (633) in pyridoxine-deficient rats The relationship of pyridoxine to protein metabolism is further strengthened by the demonstration that this vitamin is necessary for the metabolism of tryptophane When the urine of pyridoxine-deficient rats is treated with ferric ammonium sulfate, a green pigment appears (634) This substance has been identified as xanthurenic acid, an intermediary in tryptophane metabolism (635) Further studies have shown that pyridoxine is necessary for the metabolism of xanthurenic acid (636), a finding which has been confirmed in swine, in which animals the appearance of xanthurenic acid in the urine can be correlated with the onset of a characteristic anemia, which develops in this species (637) Another pigment which has been found in the acidified urine of pyridoxine-deficient swine has been identified as urorosein (638) In addition to rats and swine dogs deficient in pyridoxine excrete xanthurenic acid in the urine (639)

Based on investigations utilizing microorganisms, evidence has accumulated that the vitamin B_6 group is converted into codecarboxylase and that this substance is an important physiological form of the vitamin B_6 group Codecarboxylase functions as the co enzyme of several amino acid decarboxylases It is of interest that the codecarboxylase content of muscle and liver tissues of rats is dependent on the pyridoxine level of the diet (641) Mention should be made of the possible interrelationship between pyridoxine and the essential fatty acids (747) Skin lesions are said to appear more readily in animals deficient in the latter essentials Histological studies have not been reported, however

Pathological Effects Studies of pyridoxine deficiency have been reported on the rat (642) mouse (772) hamster (774 775), cotton rat (614) dog (647) pig (652) and monkey (296) Prominent changes have been found in the skin erythropoietic tissues and nervous tissues together with some miscellaneous lesions in other organs

Skin In the rat (642) the most prominent site of injury is the skin Grossly the initial change is an erythema of the dorsa of the paws, most commonly the hind ones This reddening soon spreads to the plantae and is followed by hyperkeratosis and scaling The digits next become swollen Coincident with these changes in the extremities the same process appears in the ears nose chin submental region and upper thorax The coat appears ill kempt but there is very little alopecia until relatively late in the course of the deficiency

Microscopically there is hyperkeratosis and acanthosis, together with erythema and edema of the corium Leukocytes are found infiltrating the

the extremities and vomiting Death ensues unless treatment is instituted Chemical studies of the blood have revealed an irregular lowering of glucose and chloride concentrations, together with an increase in non-protein nitrogen values Gross findings at autopsy have been equivocal, except for light colored livers whose fat contents on chemical analysis range from 34 7 to 55 1 percent in contrast to the normal ranges of 13-17 percent Microscopic studies have not been reported In another experiment on dogs, fatty livers and spasticity of the hind quarters have been noted but no examination of the tissues has been reported (613)

Pyridoxine

Historical In 1926 Goldberger and Lillie (623) described a 'pellagra like' condition in rats which had been placed on a diet composed in the main of cornmeal extracted with alcohol Striking lesions consisting of bilateral symmetric scaly dermatitis appeared and involved the extremities, ears and face, the trunk was only occasionally affected Since the skin changes could be prevented by autoclaved yeast these investigators assumed that the lesions were caused by deficiency of vitamin B_2 (the heat stable portion of the B group) To Gyorgy and his associates (624, 625, 626) goes the credit for showing that riboflavin (vitamin B_2 or lactoflavin) does not cure this "pellagra-like" dermatitis, and that another factor—vitamin B_6 is necessary Gyorgy suggested that the new dietary essential should be called the 'rat acrodynia factor" since the lesions of the extremities resembled those observed in human acrodynia, rather than pellagra

In 1938 a crystalline material was isolated, the hydrochloride of a nitrogeneous base (627), this had the properties of Gyorgy's vitamin B_6 and was soon shown to be 2 methyl-2 hydroxy-4 5 dis (hydroxymethyl) pyridine (628)

The synthesis of vitamin B_6 was then announced (629), and Gyorgy (603) suggested that, In accordance with the chemical nature of vitamin B_6 which is a pyridine derivative containing several oxy (methoxy) groups the term 'pyridoxine appears appropriate "

At the present time there are recognized not one but three members of the vitamin B_6 group In addition to pyridoxine pyridoxamine and pyridoxal show biological activity similar to pyridoxine at least for microorganisms (631)

Biochemical Relationships There is evidence that pyridoxine plays a role in the metabolism of protein (632) When pyridoxine deficient rats are placed on a high-protein diet (45% 30%) they develop the characteristic

latter layer The sebaceous glands and hair follicles remain unaffected until late in the disease Some observers (642) feel that these accessory structures are damaged as a result of superficial secondary infection coincident to epithelial ulceration, others, however, interpret the changes in the sebaceous glands and hair follicles as a late primary effect (317 643) The distribution of the skin lesions, particularly the initial changes in the extremities, has aroused much interest especially in relation to the distribution of the dermatitis in human pellagra No effects on the dermal lesions have been produced either by excessive sunlight or by denervation (317) It has been reported that the skin changes appear earlier in rats exposed to a cold environment (344), this is likely due to a heightened general metabolism with increased need for the vitamin rather than to any localized change in the extremities

On a pyridoxine-deficient diet the Syrian hamster is said to develop an 'acrodynia like" dermatitis about the mouth (774) Specific skin changes have not been a prominent feature of pyridoxine deficiency in the other species studied

Erythropoietic Tissues In some rats deficient in pyridoxine an anemia has been found Disturbances in red blood cell formation can be more definitely demonstrated if such animals are also rendered anemic by bleeding for then a real impairment in red blood cell regeneration develops (645) Because of this relationship of pyridoxine to hemoglobin formation in the rat, the catalase content of tissues from deficient animals has been studied, it will be recalled that this enzyme is an iron-porphyrin compound like heme No decrease in catalase content is found in the liver kidney and heart muscle of these animals (646) Such studies should be extended, however, to the dog and swine, since there is a much more marked disturbance in hematopoiesis in these species In the former (647 648, 649, 650, 651) both puppies and adult animals tend to develop anemia, which is improved by the administration of pyridoxine However normal red cell and hemoglobin levels are usually not obtained unless liver is given as well The anemia is characterized as microcytic and hypochromic, elevated plasma iron levels are observed as the blood changes progress The possible relation of folic acid to this anemia requires study

In swine an anemia is observed after animals have been on a pyridoxine-deficient regimen from four to six weeks (652) Once significant anemia appears it usually progresses in a few weeks to a severe degree, from a normal of around 8 000 000 red cells to 3 200 000 cells per cubic milliliter of blood The anemia is primarily microcytic in type Values of 40 cubic micron or less for the mean corpuscular volume have been observed (normal for swine is about 58 cubic microns) The normal mean corpuscular hemoglobin concentration of 33 percent is little if any reduced Anisocytosis is

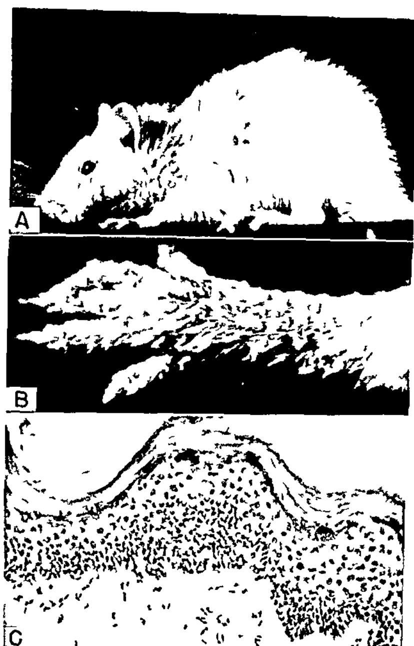


FIGURE 61 Skin Pyridoxine Deficiency (64?) *A* External appearance of rat after being on a pyridoxine deficient regimen for about six weeks Note normal appearance of fur in contrast to riboflavin deficient animal Figure 49 page 159 At this time the only change aside from some failure to grow is found in the extremities where scaling *B* is noted This appears microscopically *C*, and hyperkeratosis and acanthosis with epithelial proliferation just above the basal cell layer (Courtesy of Dr Maurice Sullivan and *The Journal of Investigative Dermatology*)

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marked, and there is an irregular reticulocytosis There is no increase in the icterus index or any increased resistance to hemolysis in hypotonic saline solution Following treatment with pyridoxine, an immediate reticulocyte response (as high as 30%) accompanied by an increase in red blood cells occurs, the cells then return to their normal size As in the dog, pyridoxine

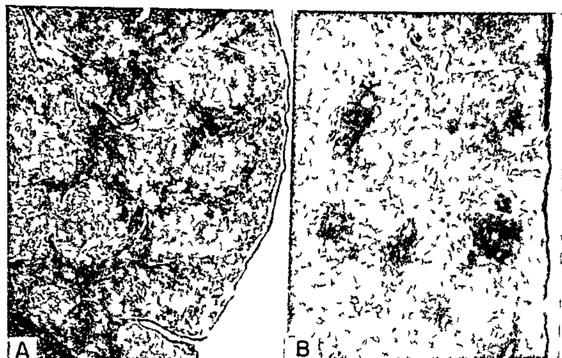


FIGURE 62 Spleen Pyridoxine Deficiency *A* Section of spleen from a pig sacrificed after being on a pyridoxine deficient diet for 139 days Significant anemia developed in 47 days and maximal anemia was present eight days before death RBC 3,650,000 MCV 43 cu μ MCHC 24 per cent (see text for normal values) The bone marrow was hyperplastic and there was hemosiderosis of this tissue as well as the liver characteristic lesions were found in the sensory neuron This section illustrates the extensive deposition of hemosiderin pigment in the pulp capsule and trabeculae Note that the malpighian bodies are spared and compare with *B* where the Malpighian bodies stand out as darker groups of cells from the lighter staining surrounding pulp No pigment is seen in this pulp there is however some pigment in the capsule This animal had been severely anemic at one time Following treatment with pyridoxine there was a sharp reticulocyte response and the blood returned to normal At autopsy no hemosiderin was found in the bone marrow liver or spleen save for the remnant of the pigment which is seen in the capsule Both Prussian blue fucsin stain x15

does not completely relieve the anemia, it must be assumed that other unknown factors possibly folic acid are necessary for hematopoiesis in swine As pyridoxine anemia develops there is a rise in serum iron content to values as high as 300 micrograms per 100 cc Upon treatment the serum iron concentration falls to the normal of 100 micrograms or less

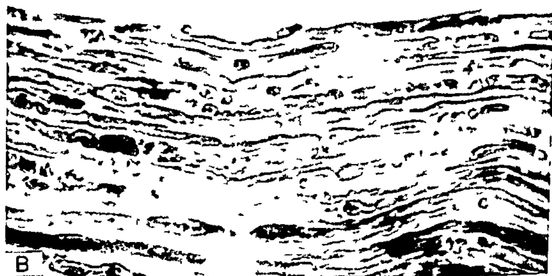
During the course of anemia in swine an extensive deposition of iron pigment (Prussian blue reaction) is found in the liver, spleen, and bone marrow This pigment, presumably hemosiderin occurs both intra- and extracellularly in the splenic pulp and in the capsule and trabeculae as well

Virtually no pigment is found in the Malpighian bodies The Kupffer cells of the liver contain pigment, and in those animals dying with severe anemia the periportal cells of the liver lobule are also filled with iron-staining material Macrophages in the bone marrow of anemic animals are loaded with pigment None has been observed in the renal tubular epithelium The bone marrow of anemic animals is hyperplastic and contains numerous "blast" cells, as well as nucleated red blood cells Treatment with pyridoxine diminishes the amount of pigment in the spleen, liver, and bone marrow, the duration of treatment can be correlated with the amount of pigment remaining in the splenic pulp Some iron-staining material remains in the capsule and trabeculae, however, even after prolonged therapy In animals receiving adequate pyridoxine there is virtually no bone marrow hyperplasia Pyridoxine anemia has been compared with that produced by phenylhydrazine and iron deficiency and shown not to result from blood destruction, since elevated serum bilirubin and increased excretion of urobilinogen in the urine and feces and of porphyrin in the urine, noted in phenylhydrazine hemolytic anemia are not observed in pyridoxine-deficient animals (653) It is concluded that the ferremia and hemosiderosis are due to the continual absorption or decreased excretion of iron at a time when its utilization for hemoglobin is at a minimum, and when the iron content of the tissues is abundant Elevated serum iron levels and hemosiderosis of the tissues do not occur in animals deficient in both iron and pyridoxine

Nervous Tissue Epileptiform fits lasting several minutes were first described in pyridoxine-deficient rats by Chick as follows (654) "1) A violent stage in which the rat would suddenly rush about wildly with protruding eyes, jumping to the floor of the room if not restrained and leaping up into the air sometimes uttering cries, this stage usually lasted less than 30 seconds In a few instances the eyes became suffused with blood, which drained away through the nasolachrymal ducts Occasionally the rat urinated during the fit and on one occasion vomiting of stomach contents was observed 2) A helpless condition in which there were muscular twitchings and tonic spasms while the rat lay helpless Sometimes the digits of one of the forepaws became clasped with those of the hind paw of the same side 3) A comatose condition when the rat sometimes became unconscious with a slowed and weakened heartbeat and absence of corneal reflex 4) Gradual recovery control being regained first of the forepart of the body and later of the hind limbs"

Similar seizures have been observed in deficient puppies but are apparently uncommon in adult dogs Pyridoxine-deficient swine show two manifestations of neurological damage during life convulsions and ataxia Convulsions may appear as early as the fourth week of deficiency, but more usually a little later—from the seventh to the twelfth week As many as three


A


B
C


C

or four attacks per day have been observed, and the "fits occur until death ensues unless treatment with pyridoxine is initiated Such 'fits" resemble those seen in the 'grand mal" of human epilepsy Attacks of shorter duration and resembling human "petit mal" have also been observed Preceding the convulsion the animal is usually excited and "nervous The pattern

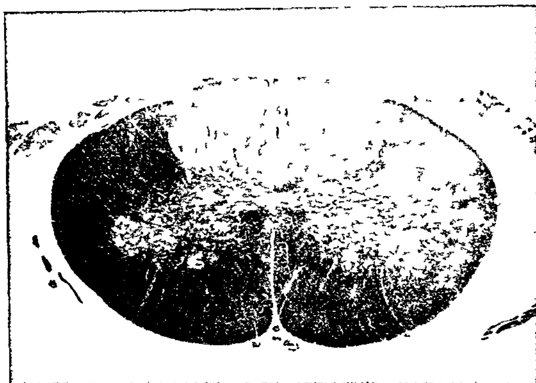


FIGURE 64 Spinal Cord Pyridoxine Deficiency Lumbar cord of same animal shown in preceding Figure Note degeneration of the dorsal columns in addition compare the differences in staining of the dorsal and ventral roots the former are much lighter than the latter indicating myelin degeneration x30 reduced 2/5 (Courtesy of the *Journal of Experimental Medicine*)

of the convulsion is as follows The pig lay on its side all four limbs and the muscles of the body jerked rapidly, the head was held in extension, the eyes shut or turned upward and saliva drooled from the mouth After several minutes the spasmodic muscular contractions ceased and a stage of stupor followed which also lasted several minutes Occasionally a gurgling sound could be heard When the stupor was over, the pig would try to get

← FIGURE 63 Sensory Neuron Pyridoxine Deficiency (611) *A* Dorsal root ganglion cells (methylene blue stain) from lumbar region from pig which had been on a pyridoxine deficient diet for 101 days Definite ataxia had been present for the past twenty days Note absence of chromotolysis (compare with Figure *A*) The cells are somewhat atrophic and occasional shrunken necrotic ones may be found *B* Sciatic nerve (modified Weigert stain) from same pig to show extensive degeneration of the myeling sheaths *C* Sciatic nerve (Bodian silver stain) which shows degeneration and fragmentation of the axis cylinders All x500 reduced 2/5 (Courtesy of the *Journal of Experimental Medicine*)

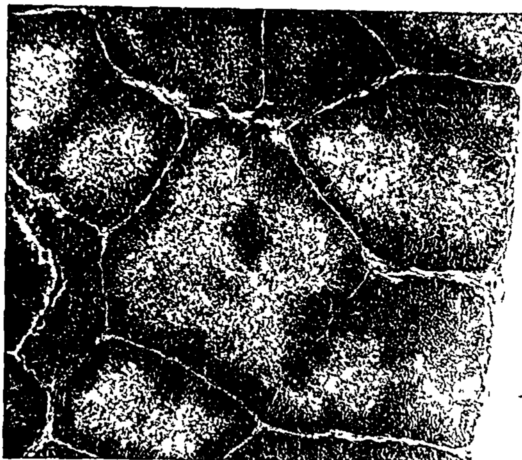


FIGURE 65 Liver Pyridoxine Deficiency *Section of liver from pig which had been on a* pyridoxine deficient diet for 113 days and was then sacrificed The animal had developed severe anemia and convulsions together with evidence of neurological involvement There is extensive fatty infiltration of the central portion of the liver lobules and deposition of hemosiderin pigment about the peripheral portion which accounts for the dark staining in this region The liver of the pig is of course normally lobulated Prussian blue-fuchsin stain ×40

up, and, when it finally succeeded it would proceed in a staggering dazed fashion" (655)

An ataxia which has been observed as early as the third week, manifests itself as a slightly high lift of the hind limbs accompanied by swaying of the hind quarters while walking There is a broad base, the legs fold under, turning in one direction or another with the result that the pig stumbles and falls *The forelegs develop similar incoordination,* and as the deficiency progresses, the animal becomes completely incapacitated

When the behavior during life is compared with the anatomical changes found at autopsy, it *appears that physiological disturbances may* be present before morphological alterations can be demonstrated The initial morphological change (611) is demyelinization of the peripheral nerves (brachial and sciatic) This is characterized by the *appearance of small droplets* of neutral fat in sections stained with Scharlach R and by vacuoles and dark

deposits in Weigert preparations Silver stains to demonstrate axis cylinders reveal questionable degeneration at this stage No alterations are detected in the dorsal-root ganglion cells As time goes on, myelin degeneration becomes more marked and there is involvement of the dorsal-root fibers and dorsal columns of the spinal cord Definite and marked axis cylinder degeneration is also found Despite these changes in the peripheral and central portions of the sensory neuron no chromotolytic alterations are encountered in the cell body Many cells become atrophic and in time, necrotic, but without the widespread dissolution of Nissl granules which is seen in pantothenic acid deficiency in swine (611)

Miscellaneous Lesions By chemical analysis an increased fat content of the liver of pyridoxine-deficient rats (656) has been reported, and a similar change has been observed histologically in swine (652) In the latter species the fat is distributed in the central areas, but in animals extremely deficient in pyridoxine lipoid accumulation also reaches the mid-zonal region In such animals fatty livers have been observed in the presence of adequate dietary choline and inositol Three of four dogs deficient in pyridoxine have been reported to develop signs of cardiac insufficiency and die suddenly (650) When adequate quantities of p-dimethylaminoazobenzene (butter yellow) are administered to rats, carcinoma of the liver develops The incidence of tumor formation may be modified by diet A reduction in the amount of dietary pyridoxine prevents the development of carcinoma In this experiment (657) a caloric effect can be ruled out since the pyridoxine-deficient animals and their controls consume the same amounts of food The effect of pyridoxine on sarcoma 180 a transplantable tumor in mice, has been studied, the removal of pyridoxine from the diet inhibits the growth of the tumor even though, as in the rat experiments the caloric intake is the same (658)

Pyridoxine Deficiency in Man A syndrome characterized by "extreme nervousness, insomnia, irritability abdominal pain weakness, and difficulty in walking" has been observed in a group of patients by Spies et al When pyridoxine is administered such symptoms disappear while nicotinic acid riboflavin, and thiamine have no therapeutic effect (659, 660) Pyridoxine has been claimed to be specific in a host of other clinical syndromes The evidence at hand, however is not convincing enough to warrant further consideration save that this vitamin appears to cure certain cases of cheilosis in which it appears to have the same curative powers as riboflavin does in others (292)

Choline

Historical The nutritional importance of choline first became apparent in 1932 when Hershey fed lecithin to depancreatized dogs which had been maintained on insulin (661) The rationale for this procedure was to determine whether the fatty liver encountered in such experimental animals could be prevented by the administration of a phospholipid Lecithin did just this and was further shown to prevent fatty livers resulting from the feeding of high fat diets to rats (662) The active principle of lecithin was soon demonstrated to be choline (663)

The effect of choline deficiency on the kidney was first described by Griffith and Wade (664) in 1939 and its relationship to hepatic damage was soon demonstrated by Gyorgy and Goldblatt (665) In the meantime Du Vigneaud (666) had elucidated the inter-relationship of choline methionine, and cystine and the phonemenon of transmethylation

Biochemical Relationships Choline is, of course an important constituent of the phospholipid, lecithin The metabolism of choline is intimately related to that of the indispensable sulfur-containing amino acid methionine When dietary choline is absent or inadequate, sufficient quantities may be formed from methionine *in vivo* to insure life (667), however, apparently not enough is synthesized to prevent certain physiological and pathological alterations in the animal organism Choline is formed *in vivo* from the combination of ethanolamine and methyl groups donated by methionine (668) ethanolamine is derived from dietary serine and glycine (669)

Metabolic studies of the liver and kidneys of rats have clarified the function of choline in the organism Ingested choline is utilized in the synthesis of certain substances which are necesasry for fat transport When choline is omitted from the diet phospholipid turnover is reduced, for instance radioactive phosphorus (P^{32}) has been utilized to show that choline stimulates phospholipid turnover in the liver and kidney, an important physiological process which must be carried on in certain organs, particularly the kidney, during critical periods of growth (670) It is assumed that the renal lesions which have been described in young rats result from a deficiency in phospholipid turnover when the needs are greatest for instance during the fourth and fifth weeks of life Choline also enhances the transportation of fatty acids from the liver to the fat depots a process which is slowed down in choline-deficient animals (671) Like methionine choline may act as a methyl donator When homocystine is fed to methionine-deficient rats, choline furnishes methyl groups to synthesize methionine a laboratory demonstration of a reaction which does not ordinarily occur in

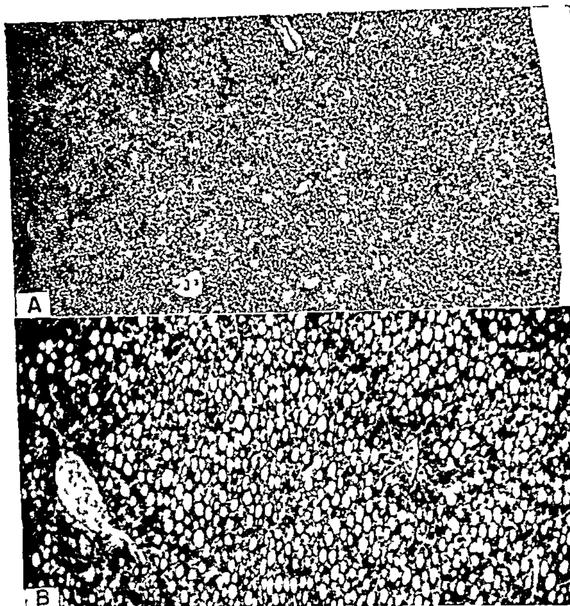


FIGURE 66 Liver Choline Deficiency *A* Low power (x40) of liver from a rat placed on a low protein (five percent) high fat (thirty percent) choline deficient diet This animal had been on this regimen for about two months Virtually every cell is distended with fat globules *B* Higher power (x150) of same section shows a periportal space and central vein to indicate the diffuse nature of the fatty infiltration H and E

the organism (271) Choline normally furnishes methyl groups to guanidoacetic acid for the formation of creatine (666)

Pathological Effects As already noted, the chief effects of choline deficiency are found in the liver and kidney Morphologic changes in the former tissues have been described in the rat, dog, and pig When young growing rats are placed on a choline-deficient low protein-high fat diet fatty infiltration of the liver rapidly occurs (665, 672, 673, 674), small sudanophilic droplets accumulate in the hepatic cells and coalesce so that

such cells become greatly distended with their nuclei pushed to one side in less than a week As others have pointed out (674) it seems quite reasonable to assume that when enough intracellular fat has accumulated the physical organization of the cell is so disrupted that metabolic changes and even necrosis may be expected to occur The latter lead to the second manifestation of choline deficiency in the liver widespread scarring That



FIGURE 67 Liver Choline Deficiency This rat had been on a low protein (five percent) high fat (22 percent) diet for over three months Note extensive fatty infiltration similar to that in the preceding Figure In addition there is scarring which divides the tissue up into lobules which have little relation to the normal architecture These connective tissue septa contain deposits of an acid fast pigment ceroid H and E ×150 (Courtesy of Dr Philip Handler and Dr I N Dubin)

the fatty change is a prerequisite for necrosis is indicated by the fact that if food intake is restricted or if dietary thiamine is deprived fat fails to appear in the liver, necrosis and scarring are also not found Grossly the chronic choline-deficient liver is pale yellow with a finely granular surface Microscopic examination reveals that the tissue is separated into irregular lobules by bands of connective tissue of varying width Usually the intact hepatic cells are infiltrated with fat

The microscopic appearance of both the rat's and dog's liver (675 676) would lead one to assume that a disturbance in hepatic function might be present Experiments in the latter species show this to be the case

In puppies choline deficiency may result in death within three weeks. Severe fatty infiltration of the liver is the only prominent manifestation of such a deficient state, livers from these animals may contain over 50 percent of lipid on a dry weight basis, which is over twice that of control animals. When bromsulfalein is administered, the dye remains in the plasma longer than normal. So too the prothrombin time is increased, and there is rise in the level of serum phosphatase. These manifestations of deranged liver function can be reversed in five to ten days if choline is administered in adequate amounts (677).

Morphologic observations of hepatic changes which follow choline therapy are inadequate. In the rat the color of the organ changes from yellow to dark reddish brown, and the size decreases. On microscepoic section a reduction in the amount of fat is observed in the cells, in addition there is evidence of regeneration of hepatic cells and large, bizarre structures containing several nuclei may be observed. As might be expected, no disappearance of the connective tissue is found at least after 6 weeks of therapy (678).

The type of diet used has an effect on the outcome of such experiments. Rations high in fat (20%) and low in protein content (4-5%) with starch as the source of carbohydrate and supplemented with cystine and cholesterol seem to be most suitable for the production of cirrhosis (674). It should be pointed out that fat deposition need not unalterably lead to necrosis, as large amounts may be found by chemical analysis in livers without necrosis as are found in those with degenerated cells. The inclusion of 0.1 percent thiouracil protects the liver against the effects of a high cystine, low casein (8%) diet (688), the reason for this is not clear.

During the development of our knowledge of the relationship of choline and the sulphur-containing amino acids to one another and to morphological changes in the liver, several groups of investigators described and studied a peculiar pigment which occurs in and about the hepatic cells of rats on choline deficient diets. Because of its waxy appearance this substance was named "ceroid" (679). An acid fast hyaline-like material, ceroid has basophilic properties, gives a negative iron reaction, is not dissolved by lipoid solvents, and exhibits a positive oxidase reaction. In contradistinction to vitamin A, its fluorescence does not fade when tissue sections are viewed under ultra-violet light (680). A number of investigators have studied the production and properties of ceroid thinking it might be related in some way to the development of hepatic damage. It was finally shown, however, that by modifying the diet cirrhosis will appear without a concomitant deposition of ceroid (681). A similar or even identical material is seen in the tissues of vitamin E deficient animals especially when large amounts of cod liver oil are administered (430). It is of interest that many of the diets which

were utilized to study cirrhosis and ceroid formation contained cod liver oil and little vitamin E Whether ceroid is a metabolic artefact or not awaits further investigation

Renal lesions in rats first described by Griffith and Wade have been inadequately studied by other investigators as well (*615, 682*) When young rats are placed on a choline-deficient diet evidence of damage to the kidney



FIGURE 68 Choline Deficiency Cortex of kidney from a weanling rat which had been on a choline-deficient diet for eight days There is an increased thickening of the capsule due to distention by red blood cells Note the focus of necrotic tubules just beneath the capsule The glomeruli are not remarkable The lumens of many tubules contain hyaline casts x150 (Courtesy of Dr Philip Handler)

appears in about 10 days It must be stressed however, that for reasons not entirely clear the development of renal lesions is not at all uniform Grossly the affected kidneys are enlarged and have a mottled reddish color On section the cortex is either diffusely dark red or a mottled red and yellow which contrasts sharply with the underlying grayish medulla Microscopically there is great dilatation of the peripheral cortical vessels, in particular, those of the capsule Hemorrhage may result from tearing of the capsular vessels due to swelling of the underlying renal parenchyma An even more prominent feature is necrosis of the renal tubular epithelium In severe cases all of these structures may be necrotic, in less damaged kidneys, the cells merely show cloudy swelling, colloid droplets, and pycnotic nuclei No stains for fat have been reported although chemical analyses have demonstrated an in-

crease in the fat content of kidneys from choline-deficient animals An attempt has been made to study the histochemical distribution of alkaline and acid phosphatase in the kidney of choline-deficient rats (683) A decrease in alkaline phosphatase does not appear until there is well marked necrosis of the tubular epithelial cells This change therefore, does not help in explaining the pathogenesis of the renal changes

The interstitial tissues are spread apart by dilated vessels a feature which depends on the amount of tubular necrosis and its duration Very little hemorrhage is present in the interstitial tissues and no red blood cells have been detected in the lumens of the tubules Pink-staining hyaline casts are plentiful however At times the glomerular tufts are dilated and the lining epithelial cells are swollen The cells of the collecting tubules appear to be normal The severity of the damage apparently determines whether an animal will or will not recover since many rats do not succumb even though continued on the choline-deficient diet In such animals the tubular epithelium regenerates to a low cuboidal type, calcification also occurs and many of the tubules become dilated When large areas of necrosis have resulted scars may be observed In such recovered organs connective tissue proliferation in the capsule is noted so that grossly the organs have a 'frosted appearance

As has been mentioned attempts to produce renal changes by means of choline deficiency in the rat at least do not yield consistent results Some of the factors which have an important bearing on the outcome have been studied Besides the content of methionine in the ration the cystine and fat composition of the diet must be mentioned Cystine has been noted to have a deleterious effect on choline-deficient animals (684 687) The reason for this is not entirely clear although it has been suggested that this amino acid promotes better growth and hence an increased requirement for choline Because of the relationship of choline-containing phospholipids to fat transport high-fat diets and variations in the saturation in the fat also have a devastating effect on choline-deficient animals (685) The protein content of the diet is another important factor when the cystine and fat content do not vary (686) Protein (casein) levels of 15% are optimal for the production of hemorrhagic changes in the kidney and when dietary protein is reduced hemorrhages appear less readily However at a level of 6 percent renal lesions do not appear in the usual 10 days, but may take 40 to 50 days to present themselves The type of dietary carbohydrate is also of importance since lesions develop when sucrose glucose and starch are the source of carbohydrate in the diet while when lactose and galactose are substituted lesions do not appear (686) In like manner the inclusion of 40 mg percent atabrine in a deficient diet protects against kidney lesions but seems to have no lipotrophic action on the liver although histological studies have not been carried out (689)

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had called attention to the deleterious effects which the feeding of unheated egg white produce in experimental animals To Boas (692) must go the credit for postulating in 1927 that egg white contains a "toxic" substance which is rendered innocuous by including in the diet certain food substances containing a protective x factor' Animals fed egg white develop dermatitis abnormal kangaroo-like posture and spasticity of the extremities During the 10 years following Boas' publication a number of investigators studied the syndrome of egg white injury and Gyorgy (693), in particular, described the histological changes in the skin and demonstrated a curative factor from certain foodstuffs, this factor was designated vitamin H In the meantime workers in other fields were providing information which was to clarify the problem A new factor named coenzyme R had been described as an essential for legume nodule bacteria in 1933 (694) A little later a crystalline material Bios II, was shown to be necessary for the growth of yeast cells (695) In 1939 the suggestion was made that coenzyme R and Bios II were identical (696) and a year later du Vigneaud and his associates (697) proved that Gyorgy's vitamin H, coenzyme R and Bios II were one and the same substance In 1942 du Vigneaud (698) announced the structure of biotin, an active substance was soon synthesized (699) and was found to elicit the same physiological responses as the natural product

While the structure of this vitamin was being elucidated an active antibiotin principle from egg white was crystalized, this material is called avidin (700, 701)

Biochemical Relationships Little is known of the functions of biotin in the animal organism The possible relationship to the metabolism of lactate and pyruvate has been recently advanced when liver slices from biotin deficient animals are studied *in vitro* they exhibit a 25 to 35 percent increase in the disappearance of lactate when the biotin is added to the medium (702) Whether this is an effect of inanition remains to be determined

Pathological Effects Biotin has been shown to be a dietary essential for the rat the mouse the hamster, dog and monkey Skin lesions have been described in several of these species, in addition studies of the nervous tissues and muscles have been made The role of biotin in tumor formation has also been investigated

Skin Sullivan and Nichols (703) have placed young rats on a diet containing 30 percent dried egg white and find that gross and microscopic cutaneous lesions develop after 3 to 5 weeks The initial change is generalized erythema the coat becomes roughened and loses its luster A generalized scaling follows which is accompanied by a symmetrical alopecia first developing over the chin, neck, and anterior portion of the venter and spreading to the rest of the body surface Such rats which are covered with brown greasy scales, are not particularly pleasant sights

Certain other manifestations of choline deficiency should be mentioned. Hemorrhages may be encountered in the eyes of choline-deficient rats (690); free blood is found between the anterior limiting membrane of the vitreous and the crystalline lens and the ciliary process is swollen and hemorrhagic. Hemorrhages may also appear in the glomerular layer of the adrenal glands and necrosis of the cortical cells has been sometimes noted as well. So too foci of hemorrhage and necrosis of muscle fibers have been observed in the heart, while extensive intracranial hemorrhages have been described in some of the young born to choline deficient females (213) although vitamin K deficiency was not ruled out in this connection. In view of the fact that increased prothrombin times have been observed in choline deficient dogs (675) a possible explanation of the hemorrhages particularly those in the kidney of the rat may be due to lowered prothrombin values in the blood resulting from damage to the liver as a result of fatty infiltration.

Choline Deficiency in Man. Excessive fat accumulation in the liver is not uncommon at autopsy. In the absence of obstruction of the pancreatic ducts the question arises as to whether such fatty infiltration is related to any dietary factors. It has been known of course that simple starvation leads to abnormal accumulation of fat in the liver. With the development of our knowledge of the hepatic lipotropic action of methionine and/or choline, the possibility that fatty livers in the human might result from deficiencies in these factors has received much interest. These materials in addition to the B group of vitamins are being used clinically in the treatment of liver disease.

It is doubtful whether choline deficiency *per se* can occur in man. However, it is not unlikely that a deficiency of this material and its precursor methionine can result when dietary protein is inadequate. In the chronic alcoholic whose daily caloric requirements may be satisfied by 500 cc of alcohol such a situation could easily arise. It is not surprising that choline and its precursor methionine in the form of high protein intake with additional vitamins of the B group are being used to treat fatty liver and cirrhosis especially in alcoholics and those with poor dietary histories (753). One interesting report has appeared dealing with another form of choline therapy (691). This was a case of addisonian pernicious anemia refractory to liver therapy. Liver biopsy proved that the patient had an extremely fatty liver. Intravenous choline chloride returned the liver and blood picture to normal.

Biotin

Historical. Knowledge of biotin developed along three independent lines of research. Since the beginning of the century a number of investigators

Biotin deficient hamsters (705) develop a dermatitis at the corners of the mouth, such lesions spread more excessively as the deficiency progresses. Alopecia occurs in biotin deficient mice (780). Dermatitis and alopecia have been described in rabbits fed a diet containing excessive amounts of egg white (706). In monkeys (707) rendered acutely deficient in biotin, a scaling dermatitis has been described, this appears most conspicuously over the face, arms and legs. In more chronically deficient animals, the hair becomes thin and loses its color. In none of these last four species have histological studies been reported.

Nervous Tissues and Muscles. Because of the peculiar attitude and gait of biotin-deficient rats the nervous tissues and muscles of deficient animals have been studied (704). Careful examinations of the fore brain, the hind brain, the spinal cord, posterior root ganglia and sciatic nerve have failed to reveal any abnormality. Studies of muscle tissues however have demonstrated atrophy, necrosis of fibers, and an increase in sarcolemma nuclei similar to the changes which are observed in alpha-tocopherol deficiency. When large doses of the latter vitamin were added to the diet of a second group of animals only atrophy was found so that it appears that biotin is not the etiological factor responsible for the changes described in the first series of animals. A single myographic reading following stimulation of the sciatic nerve gave no evidence of repetitive discharge, this would indicate that the rigidity which is observed is not myotonic in origin. In view of the physiological changes which appear in the muscle, it is of interest to note that higher creatine contents of sciatic muscle from deficient animals have been noted than in controls, it is unfortunate however, that comparison was not made with inanition controls (708). Studies of other tissues of biotin deficient rats have not revealed any other significant morphological changes, there is however some evidence that biotin deficiency leads to an anemia in dogs (711) but not rats (712).

Tumors. When biotin is added to diets which ordinarily protect rats from developing hepatic tumors produced by feeding, a decrease in the protective value of the diet occurs (710). In other words biotin appears to act as an anti inhibitor of the growth of this type of neoplasm in rats. As might be expected this finding has led to the therapy of human cancer with avidin but thus far the administration of egg white or avidin to humans with malignant tumors has not met with encouraging results.

Biotin Deficiency in Man. Biotin deficiency has been produced in experimental subjects to whom 200 grams of dehydrated egg-white was fed daily (713). After three to four weeks on such a diet all four volunteers developed a fine, non pruritic scaling of the skin. Although the dietary regimen was maintained the skin lesions disappeared after a time. One subject developed a maculosquamous dermatitis of the hands, arms and legs



FIGURE 69 Skin Biotin Deficiency (703) *A* External appearance of a rat which had been placed on a diet containing thirty per cent egg white There is complete absence of hair and the entire body is covered by greasy yellow scales Note also the humped position and gait *B* Skin from late stage similar to that depicted grossly Note hyperkeratosis with dilatation of the orifices of the hair follicles This results in a peculiar finger like appearance of the epithelium There is relatively little change in the underlying corium (Courtesy of Dr Maurice Sullivan and the *Archives of Dermatology and Syphilology*)

Microscopically the skin shows an increasing hyperkeratosis, together with a uniform acanthosis or increase in the prickle cell layer A sparse but definite diffuse cellular infiltration of the corium then appears, this latter structure also becomes edematous The shafts of the hair follicles then appear dilated and their patulous orifices become clogged with hyperkeratotic sudanophilic material By the late stage of the disease the epidermis is atrophic, there is a diminution in the size of the sebaceous glands and small superficial ulcers and epidermal atrophy appear The meibomian glands are not affected Biotin concentrates completely ameliorate these cutaneous changes

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All evidenced a peculiar grayish color of the skin and all ultimately showed atrophy of the lingual papillae. Anorexia, extreme lassitude, sleeplessness and muscle pain were also accompaniments of the deficient state. Two subjects complained of precordial distress, and electrocardiographic alterations were present. Biotin therapy afforded prompt relief of these signs and symptoms. Spontaneous biotin deficiency in man seems extremely remote since balance studies indicate that enough biotin is synthesized by the intestinal flora and is absorbed in large enough quantities so that the human does not need an exogenous source of the vitamin (714). In addition, it is unlikely that the ordinary diet could contain enough of the anti-biotin avidin to lead to biotin deficiency.

Folic Acid (*L. Casei* Factor)*

Historical. In 1935 Day and his associates (715) described a syndrome consisting of anemia, leukopenia, necrosis of the gums and diarrhea in monkeys, signs developed on a diet deficient in the B group other than thiamine. Since further studies eliminated riboflavin and nicotinic acid as the causal factors of the changes exhibited by such monkeys (716), an unknown substance present in liver and yeast was designated as the active principle or vitamin M. Day and his collaborators then showed that anemia and leukopenia in the monkey can not be cured by the inclusion of pyridoxine and calcium pantothenate with other crystalline vitamins and that anemia can be prevented by a concentrate of folic acid (717). A little later Day demonstrated that purified *L. Casei* Factor is effective in curing the anemia of deficient monkeys (718).

While these investigations on the monkey were being carried out Sebrell et al. (719) had shown that folic acid cures granulocytopenia in rats whose diets contained sulfaguanidine. During the same period the factors referred to above had been isolated and shown to be indispensable for certain microorganisms such as Lactobacillus casei and Streptococcus fecalis, hence the terms "*L. Casei* Factor" and "Folic Acid," as others had called a similar active compound derived from leafy vegetables (720). For several years the nature and interrelationships of these materials were not entirely clear. However, in 1945 the synthesis of an active *L. casei* factor was reported (721) and during the latter part of that year and the early months of 1946 a number of clinical reports appeared which demonstrated the importance of this synthetic material in the treatment of various types of macrocytic anemia in the human. The structure of this synthetic material was finally announced in 1946 (722), to be made up of 3 substances: a 2 ringed nitrogen compound

* Pteroylglutamic Acid

or a pteridine, para-aminobenzoic acid and glutamic acid. The latter is present in varying amounts depending on the natural source from which the active compounds are derived.

Biochemical Relationships. Little is known of the chemical reactions in which folic acid participates. Studies on humans have demonstrated that this material converts the megaloblastic bone marrow, which is observed in pernicious anemia and other macrocytic anemias, to a normoblastic type of tissue. The mechanism for this transformation is, of course, not understood. At this writing there is no clear-cut evidence of the relationship of folic acid to the intrinsic factor, extrinsic factor, or the erythrocyte maturing factor. Studies of clinical cases of macrocytic anemia already reported would seem to indicate that folic acid is not identical with the first two factors and from amounts which must be administered in order to produce therapeutic effects, it is unlikely that folic acid is the EMF factor of liver extract, either.

Pathological Effects. The earlier studies of Day (715, 716) helped very little to characterize the blood dyscrasia which appears in monkeys. Such animals display moderate anemia and leukopenia; no examination of the bone marrow or other tissues was reported. So too, the tissues of Doan's animals were not investigated and only the blood picture has been reported upon (724).

The group at the National Institute of Health reported the effects on rats of sulfaguanidine or sulfasuxidine (1%) incorporated into purified diets (723). Severe leukopenia and agranulocytosis are produced by this means as well as reduction of hemoglobin concentrations in some animals. The marrow of rats showing granulocytopenia exhibits a decreased number of the cells of this series, particularly adult and juvenile forms. In some rats there is evidence of impaired erythropoiesis. The peripheral blood usually shows a reduction in red cell count and hemoglobin concentrations. There is, of course, a reduction in white blood cells. For instance, one rat (14651) receiving 1% sulfaguanidine in the diet for 27 days had a hemoglobin concentration of 8.5 Gms. and a white count of 2,650 of which only 6% were granulocytes. After 57 days of therapy with liver, the hemoglobin had risen to 14.0 Gms. and the white count to 6,600 of which 26% were granulocytes. It is unfortunate that the results reported are not very consistent, that is, the development of anemia appears to be extremely variable though the effects of the deficiency and therapy on white blood cells are more uniform. This adverse hematological response to sulfonamides in the diet may be reversed by the administration of crystalline folic acid (719). The granulocytopenia and leukopenia are apparently corrected; evidence for an effect on anemia appears to be less convincing. It is unfortunate that this anemia has not been characterized, that is, we do not know whether it is macrocytic in type or not.

L. Casei Factor Deficiency in Man. Because of the response of rats and monkeys to *L. casei* factor or to "folic acid" it is not surprising that the antianemic property of this material has been investigated in man. Soon after the announcement of the preparation of synthetic *L. casei* factor groups of investigators in Birmingham (725, 726), Nashville (727), St. Louis (728), Columbus (729), and Detroit (730) announced the efficacy of this material in unspecified nutritional macrocytic anemia, tropical macrocytic anemia, sprue, addisonian pernicious anemia, pernicious anemia of pregnancy, and macrocytic anemia of infancy.

In such cases the synthetic compound is effective when given orally, parenterally, or intravenously. It is not clear as yet whether *L. casei* factor is the anti-pernicious anemia factor or not. At any rate in the above types of macrocytic anemia it readily leads to a pronounced reticulocyte response, a rise in hemoglobin, and an increase in leukocyte count so that "it becomes increasingly apparent that we are dealing with one of the more fundamental molecules essential to the normal metabolism of all cell types in marrow, and in young actively growing cells and tissues generally, perhaps" (729). In addition the above studies which were referred to would seem to indicate that lingual changes, when present, are ameliorated. Insufficient data are available on the effect of this compound or the nervous tissues of addisonian pernicious anemia.

Inositol

Historical. Although inositol was isolated from living tissues during the last century, *its designation as an essential nutrient did not come* until 1940 when Woolley (731) described alopecia in mice which had been placed on a diet deficient in this substance. Since Woolley's report both positive and negative experimental results have been recorded. At the present time, however, the essential nature of dietary inositol seems established. Variations in biological response, which have been so confusing, are likely due to differences in bacterial synthesis of the material by the gastrointestinal flora.

Biochemical Relationships. Inositol is a constituent of a phosphatide derived from brain and as such may function in a fashion similar to choline (732). Its relationship to fat metabolism has been shown by its protective action on fatty infiltration on the liver (686, 733), although it appears to enhance the incidence and severity of renal lesions on a choline deficient-low protein diet (686). Beef heart is a rich source of inositol, containing 1.6 percent on a dry *weight basis* (735). The significance of this remains to be determined.

Pathological Effects No histological studies have been reported on animals depleted of inositol Positive results dealing with its effects on growth have been reported in mice (731) rats (736) cotton rats (614) and possibly hamsters (705)

Inositol Deficiency in Man Inositol has been shown to decrease liver fat ordinarily found in human patients with gastrointestinal carcinomata (737) this is the only observation reported on a positive action by this material on man



FIGURE 70 *Inositol Deficiency Mouse which had been placed on an inositol deficient diet Note loss of hair over body with fur still remaining over head and extremities (Courtesy of Dr D W Woolley)*

Para-Aminobenzoic Acid

Historical The possibility that para-aminobenzoic acid is an indispensable nutrient was advanced by Ansbacher (738) in 1941

Biochemical Relationships At the present time there are virtually no data on the possible role of para-aminobenzoic acid in biological processes save that it is a part of the folic acid molecule (722)

Pathological Effects Ansbacher (738) has been able to produce achromotrichia in rats on a synthetic diet and to restore the color of the fur with para-aminobenzoic acid The achromotrichia is apparently unrelated to pantothenic acid or copper deficiencies (pages 176 and 52) No studies



FIGURE 71 Achromotrichia Para aminobenzoic Acid Deficiency *A* Rat which had been on a para aminobenzoic acid deficient diet for four weeks *B* Rat on same deficient diet for four weeks and then two weeks on the same diet supplemented with three mg of para amino benzoic acid per day (Courtesy of Dr S Ansbacher)

have as yet been reported on the tissues of para-aminobenzoic acid deficient animals

PART V

THE ESSENTIAL FATTY ACIDS

If these well known fatty acids (in lard) are responsible for the cures described then we must assign to them a function far more subtle than the production of nine calories of energy per gm burned By their presence they have changed the entire economy of the animal, causing an increase in body weight equal to 10 times the weight of the acids consumed The increase in weight is always accompanied by a return to normal health ' Burr and Burr 1929 (739)

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Historical In 1929, Burr and Burr (739) described a new disease in rats which had been placed on a fat-free diet This chronic disease was characterized by growth failure after 4 or 5 months and skin and kidney lesions it could be cured by as little as ten drops of lard each day These investigators soon showed that the specific factor was linoleic acid which was therefore designated as an essential fatty acid" (740) Linolenic and arachidonic acid have since been shown to substitute for linoleic acid at least as far as growth is concerned

Biochemical Relationships The physiological function of the essential fatty acid is obscure Schonheimer (741) has demonstrated that these substances in contrast to other fatty acids are not formed within the organism Unsaturated fatty acids seem to be related to the deposition of ceroid (page 127 Fatty acid deficiency in the rat is accompanied by an increased consumption of water, the average daily consumption of deficient and control animals has been reported as 20 9 cc and 1 5 cc respectively (740)

Pathological Effects As yet the effects of fatty acid deficiencies have only been reported in the rat and dog and in both these species the reports have only been fragmentary

Inadequate histological studies have been reported on the skin (742) kidneys (743), and reproductive tissues (744 745) of rats When such animals are placed on a fat-free diet the initial change which occurs in from 70 to 90 days, is scaling of the epidermis over the dorsa of the feet Scaling of the tail also occurs and the tip usually becomes inflammed and sometimes necrotic Alopecia of the head neck and back has also been observed Skin lesions are affected by the degree of humidity of the environment being made more severe by a low relative humidity (747) Microscopic examination of the skin has revealed hyperkeratosis, but no mention has been made of the appearance of the hair follicles and sebaceous glands (742)

Bloody urine and kidney lesions are important manifestations described by the Burrs and renal damage is said to be more severe on a high protein diet Grossly the organs are enlarged and pale with finely pitted and coarsely granular surfaces (743) The pathogenesis of the renal lesions is not at all clear The principle site of damage is the tubular epithelium The cells are filled with lipoid Some are necrotic The tubular lumen contains hyaline material and fat droplets Casts of similar composition are found in the collecting tubules as well Calcification is prominent following damage to the tubular epithelium In some of the animals tubules lined by flat regenerated epithelium are found The glomeruli are said to be normal in all animals The cells of the collecting tubules also contain sudanophilic material and are necrotic The papillae are calcified An illustration depicts necrosis,

PART V

THE ESSENTIAL FATTY ACIDS

PART VI

THE PATHOLOGIC ANATOMY OF SPECIFIC TISSUES

A RECAPITULATION AND COMPARISON

In the foregoing sections of this book, specific tissue changes resulting from deficiencies of each of the essential inorganic elements amino acids vitamins, and fatty acids were discussed Alterations have been described in a variety of organs and tissues, in fact, as a glance at Table VI will demonstrate, virtually every mammalian tissue may be affected For convenience sake, Table VI has been arranged on a morphological not etiological basis It is hoped that such an approach to nosography will be pardoned All of the changes which are listed have been discussed in the preceding sections

Such a wide variety of lesions furnishes the histologist and histochemist potent techniques with which to study both morphological and physiological disturbances in cells and tissues In the following pages an attempt will be made to recapitulate the changes which may be produced by deficiencies in specific nutrients, in addition certain instances will be mentioned in which comparative studies should prove fruitful

apical degeneration and calcification" of a papilla which, however, resembles more nearly artefact produced by tearing of calcified material. Calcification in this region appears to be both inter- and intratubular. In the pelvis "hyperplasia of the epithelium" has been described. Such epithelium is not cornified. *The choline and protein content of the diet employed is not clear* and these, of course, are especially important in view of the lesions which have been described in choline deficient rats (page 195).

Disturbances in reproductive activity have been described and histological studies have been reported in male (744) and female (745) animals. In no studies has the paired-feeding technique been used, so that it is quite possible that the changes described are merely due to inanition. In the male animal there is a loss of sex interest and macroscopic atrophy of the testes. Microscopically the tubular epithelium shows various degrees of degeneration and characteristic giant cell formation. Regeneration takes place following administration of an essential fatty acid.

In the female animal reproduction is affected early, ovulation late in the course of a deficiency. There are atrophic changes and under-development of the uterine mucosa and maternal decidua. The embryos are either resorbed or remain in utero longer than normal. Hemorrhage and necrosis accompanied by secondary inflammatory phenomena have been observed in the placentae and uterine walls. Changes which have been described are reminiscent of those which have been reported in vitamin A deficiency in rats.

In the dog a 'scaling flaky desquamation' has been produced by a fat-free diet. In addition the hair becomes dry and coarse (746). No histological studies have been reported.

Fatty Acid Deficiency in Man. A series of children with eczema and other diseases has been studied by Hansen (747). In the eczema group there was a significant lowering of the iodine number of the fatty acids of the serum. When large doses of oils having high iodine numbers were administered the serum iodine number rose and there was a coincident improvement in the skin lesions.

Part VI

THE PATHOLOGIC ANATOMY OF SPECIFIC TISSUES

A RECAPITULATION AND COMPARISON

In the foregoing sections of this book, specific tissue changes resulting from deficiencies of each of the essential inorganic elements amino acids vitamins, and fatty acids were discussed. Alterations have been described in a variety of organs and tissues, in fact, as a glance at Table VI will demonstrate, virtually every mammalian tissue may be affected. For convenience sake, Table VI has been arranged on a morphological not etiological basis. It is hoped that such an approach to nosography will be pardoned. All of the changes which are listed have been discussed in the preceding sections.

Such a wide variety of lesions furnishes the histologist and histochemist potent techniques with which to study both morphological and physiological disturbances in cells and tissues. In the following pages an attempt will be made to recapitulate the changes which may be produced by deficiencies in specific nutrients, in addition certain instances will be mentioned in which comparative studies should prove fruitful.

apical degeneration and calcification' of a papilla which, however, resembles more nearly artefact produced by tearing of calcified material. Calcification in this region appears to be both inter- and intratubular. In the pelvis 'hyperplasia of the epithelium" has been described. Such epithelium is not cornified. The choline and protein content of the diet employed is not clear and these, of course, are especially important in view of the lesions which have been described in choline deficient rats (page 195).

Disturbances in reproductive activity have been described and histological studies have been reported in male (744) and female (745) animals. In no studies has the paired-feeding technique been used so that it is quite possible that the changes described are merely due to inanition. In the male animal there is a loss of sex interest and macroscopic atrophy of the testes. Microscopically the tubular epithelium shows various degrees of degeneration and characteristic giant cell formation. Regeneration takes place following administration of an essential fatty acid.

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Table VI (continued)

4) TEETH
  a) *Dentine* Calcium phosphorus vitamin D ascorbic acid
  b) *Enamel*
    Enamel Organ Magnesium fluorine (?) vitamin A
    Pigmentation (rat) Iron tryptophane vitamin A alpha tocopherol

BLOOD FORMING TISSUES VESSELS AND THE COAGULATING MECHANISM

1) RED BLOOD CELLS
  a) *Erythropoiesis* Copper cobalt nicotinic acid (?) folic acid
  b) *Hemoglobin Formation* Iron copper pyridoxine tryptophane

2) WHITE BLOOD CELLS Folic acid

3) PLATELETS Folic acid

4) BLOOD VESSELS Calcium (?) ascorbic acid vitamin K (?)

5) CLOTTING MECHANISM Calcium vitamin K

MUSCLE TISSUES

1) HEART Potassium alpha tocopherol thiamine

2) SKELETAL MUSCLE Alpha tocopherol

3) SMOOTH MUSCLE Alpha tocopherol (pigment)

NERVOUS TISSUES

1) PERIPHERAL NERVE Pyridoxine pantothenic acid riboflavin (?) nicotinic acid (?)

2) BRAIN Copper thiamine (?)

Table VI

SPECIFIC TISSUE CHANGES ASSOCIATED WITH DEFICIENCIES OF ELEMENTS, AMINO ACIDS, VITAMINS, AND FATTY ACIDS

EPITHELIAL TISSUES

1) SKIN
   a) *Epidermis* Magnesium, zinc, vitamin A, riboflavin, pantothenic acid, pyridoxine, biotin, linoleic acid
   b) *Hair Follicle and Hair Shaft*
      Alopecia Zinc, tryptophane, riboflavin, pantothenic acid, biotin, inositol
      Achromotrichia Copper, pantothenic acid, para-aminobenzoic acid
   c) *Sebaceous Glands* Zinc, riboflavin

2) GASTRO-INTESTINAL TRACT
   a) *Buccal Cavity* Zinc, riboflavin, nicotinic acid (?)
   b) *Salivary Glands* Vitamin A
   c) *Esophagus* Zinc
   d) *Stomach* Calcium
   e) *Intestine* Pantothenic acid

3) EYE AND PARAOCULAR GLANDS
   a) *Cornea* Sodium, zinc, tryptophane, lysine, histidine, riboflavin, vitamin A
   b) *Conjunctiva* Sodium, vitamin A
   c) *Lens* Calcium, tryptophane, riboflavin
   d) *Globe and Retina* Choline, vitamin A
   e) *Lacrimal Glands* Vitamin A
   f) *Tarsal Glands* Sodium
   g) *Harderian Glands* Pantothenic acid

4) LIVER Magnesium, methionine (cystine, choline), riboflavin, pantothenic acid, pyridoxine, inositol

5) PANCREAS Vitamin A

6) ADRENAL Pantothenic acid

7) GENITO-URINARY TRACT
   a) *Kidney* Magnesium, potassium, chlorine, choline, linoleic acid
   b) *Pelvis, Ureter, Bladder* Vitamin A
   c) *Testis* Alpha-tocopherol
   d) *Accessory Male Sex Organs* Vitamin A
   e) *Ovary and Reproduction* Alpha-tocopherol
   f) *Accessory Female Sex Organs* Vitamin A

8) RESPIRATORY TRACT
   a) *Trachea and Bronchi* Vitamin A

9) THYROID Iodine

10) PARATHYROID Calcium

11) HYPOPHYSIS Leucine (?)

MESENCHYMAL TISSUES

1) CONNECTIVE TISSUE Ascorbic Acid

2) CARTILAGE Calcium, phosphorus, vitamin D

3) BONE Calcium, phosphorus, manganese, vitamin A, vitamin D

Part VI

THE PATHOLOGIC ANATOMY OF SPECIFIC TISSUES
A RECAPITULATION AND COMPARISON

Table VII

SPECIFIC CHANGES IN EPITHELIUM
PRODUCED BY DEFICIENCIES OF ESSENTIAL NUTRIENTS IN RATS

TYPE OF DEFICIENCY	GROSS DISTRIBUTION AND APPEARANCE	EPITHELIUM	HAIR FOLLICLES	SEBACEOUS GLANDS	CORIUM
MAGNESIUM (60)	Erythema of paws and ears	Hyperkeratosis late	Intact	Intact	Dilated vessels
ZINC (185)	Alopecia of dorsum with scaling	Hyperkeratosis acanthosis and parakeratosis	Atrophy	Hypertrophied	Hyperemic late
RIBOFLAVIN (552)	Alopecia of head venter abdomen	Atrophy	Atrophy	Necrosis	Atrophy
PYRIDOXINE (642)	Symmetric scaling dermatitis of paws ears nose shin chest edema of paws	Hyperkeratosis and acanthosis	Intact until late	Intact until late	Edema hyperemia and inflammation
PANTOTHENIC ACID (606)	Alopecia of venter scaling of paws graying of fur	Hyperkeratosis acanthosis and vesiculation	Dilatation	Intact until late	No characteristic chang
BIOTIN (703)	Generalized scaly, greasy dermatitis with alopecia	Extreme hyperkeratosis and acanthosis	Dilated and plugged	Intact until late	Intact until late
VITAMIN A (374)	Lesions only in atrophic skin	Keratinization	Keratinization	No change	No change
LINOLEIC ACID (742)	Scaling of dorsae of feet necrosis of tip of tail	Hyperkeratosis	?	?	?

Epithelial Tissues

Skin

As one of the most frequently involved sites in nutritional deficiency disease and as one which has been studied rather extensively, the skin presents an excellent example of the divergent pathological alterations which may occur. The lesions which have been reported thus far, particularly those occurring in the rat, exhibit a tremendous variability with regard to differences in involvement of the epithelium and its appendages, the hair follicles and sebaceous glands; in addition there are individual peculiarities of distribution over the body surface. Studies of nutritional dermatoses in the rat by Sullivan and others have placed a number on such a secure pathological basis that it is fairly easy to differentiate one from another when the general distribution and the histological changes are understood. Our knowledge of lesions in species other than the rat is only fragmentary, so that much further study will be necessary before these are understood. What is to follow should provide a stimulus for investigations of nutritional dermatoses in other animals; this should prove a tremendous boon to comparative dermatology.

The accompanying Table VII summarizes the alterations which have already been described in the rat in some detail. Individual differences in the distribution, gross appearance, and the microscopic changes in the epithelium, hair follicles, sebaceous glands, and corium of rats deficient in any one of the eight nutrients listed will be noted.

Gross Distribution and Appearance. One of the most striking characteristics of the distribution of these skin lesions in rats is their wide variability; for instance, the change may be generalized or only certain specific areas of the body, such as the extremities, may be involved, at least in the earlier stages. The reason for this is not clear; variations in the metabolism of skin from different regions of the body might furnish an answer. *In vitro* studies of the metabolism of skin from different sites in normal animals should be compared with studies of those deficient in the various essential nutrients. So too, the effects of physical agents such as light, heat, humidity, et cetera, might bring out interesting data such as those which have already been obtained with carcinogens (14). The response of normal skin and that from vitamin-deficient animals to a large number of injurious agents must be studied.

Epithelium. In the epidermis hyperkeratosis and acanthosis are the most prominent manifestations of dermal involvement; parakeratosis is infrequent. The significance of these changes is not clear but fundamentally they would seem to indicate some defect in the normal maturation and differ-

Skin Changes in Man In several of the human deficiency syndromes skin lesions are prominent It is not always clear, however, which nutrient is responsible for the observed changes In experimental ascorbic acid deficiency a perifollicular, papullar hyperkeratosis has been observed (465) and cleared up following vitamin C therapy Such lesions resemble grossly changes which were thought to be specific for human vitamin A deficiency (323) However the specificity of these lesions has recently been questioned (324) and a relation to deficiency of the B group suggested The situation is, therefore, much confused Lesions at the angles of the mouth (cheilosis) which were thought to be specific for riboflavin deficiency (586) have been cured by pyridoxine (292)

Gastro-Intestinal Tract

Buccal Cavity The lining of the base of the tongue exhibits parakeratosis in zinc-deficient rats (185) while the lingual filiform papillae show defective formation of cornified cells in riboflavin-deficient animals (552) Lesions of the gums and tongue are of course a characteristic part of the blacktongue syndrome in dogs but inasmuch as such changes can be produced only with difficulty when animals are placed on a purified diet lacking nicotinic acid the relationship of this substance to the lesions encountered is not clear at the present time (588) The tongue must be investigated more carefully in rats and other species subjected to deficiencies of essential nutrients When one recalls the extensive lingual changes which are seen in human pellagra and in pernicious anemia it is extraordinary that so little study has been devoted to the tongue of experimental animals The tongue in the human is somewhat similar to the cornea of the rat Deficiencies of various nutrients riboflavin nicotinic acid iron and the E M F all seem to affect the lingual epithelium just as riboflavin lysine zinc and sodium do the rat s cornea

Salivary Glands These structures are involved in vitamin A deficiency (312) There is metaplasia of the ducts which leads to blockage and atrophy of the glandular epithelial cells A deficiency of no other essential nutrient has as yet been shown to produce changes in the various salivary glands

Esophagus A most unique alteration has been reported in the esophagus of zinc-deficient rats (185) in which there is thickening of the epithelium due to the presence of partially keratinized cell layers Similar or any changes in fact have not been described in other deficiency syndromes though it is unusual to find experiments in which this structure is said to have been examined

Stomach Ulcers of the stomach have been said to result from a number of deficiencies of necessary nutrients in many instances such diets have lacked other essentials so that the specificity of the lesions must be ques-

entiation of the epithelial cell and as such of course, are *indications of a deranged metabolism* Such changes are not seen in all of the deficiency syndromes, however, as atrophy of the epidermis is found in riboflavin-deficient rats (522) while those placed on magnesium-deficient rations have an entirely normal superficial covering until late in the course of the deficiency (60)

Hair Follicles and Hair Shaft In certain deficiency syndromes in the rat, such as those produced by zinc (185) and riboflavin (522), there is atrophy of the hair follicles and alopecia In others such as pantothenic acid (606) and biotin (703) dilatation of the orifices of the hair follicles with loss of hair is prominent When vitamin A deficiency is induced in the presence of an atrophic hair follicle there is keratinization of the follicular epithelium (324) Other deficiencies have not been well enough studied to determine the specific changes, if any which may occur in the follicle itself, these include copper (137) and para-aminobenzoic acid (738), in which there is achromotrichia and *inositol* (731) and tryptophane (231) deficiencies in which there is alopecia Understanding of the pathogenesis of the dermal changes in fatty acid deficiency is likewise very inadequate (742) The interrelationship of copper (137) pantothenic acid (606), and para-aminobenzoic acid (738) to a loss of color of the hair should be investigated On a morphologic basis the achromotrichia produced by copper and pantothenic acid deficiencies appears to be different (137) Furthermore studies of positive chromotrichia factors such as phenylthiocarbamide (749) in relation to the above nutrients should furnish data which may be of great interest

Sebaceous Glands Although these structures may also show alterations such as atrophy or hypertrophy in riboflavin or zinc deficiencies in the majority of the deficient states thus far studied the cells composing these glands tend to remain intact and exhibit no specific morphological changes

Corium Alterations in the corium are usually not very prominent until late in the course of a deficiency when secondary alterations, as a result of infection, are seen Vascular disturbances however, manifested by dilatation of the blood vessels may be observed in animals deficient in magnesium (60) pyridoxine (642), or in the pellagra syndrome The cause for such changes is not clear

The few pointed investigations on the effect of multiple deficiencies on the skin of rats have been referred to before (page 11) This field would seem to be a fruitful one for further investigation So too the relation of hormones to changes produced by nutritional means should be of interest, for instance, the administration of large amounts of estrogen to rats (7) leads to changes in the skin which seem to be similar to those found in riboflavin-deficient animals (552) whose livers cannot inactivate estrogen *in vitro*

lens is an extremely sensitive tissue to disturbance in metabolism so that it is not unlikely that other deficient cataracts will be described

Eye Bulb and Retina Aside from the lesions of the cornea and lens which have just been mentioned, little else has been observed in the eye bulb itself Hemorrhages have been noted in and about the ciliary body of a few choline-deficient rats (690) Extensive degeneration of the retina is said to occur as a result of vitamin A deficiency (322) It is apparent, however that this portion of ocular apparatus has not been extensively studied in experimental animals and it is likely that other lesions will come into prominence when it is more carefully investigated Especially interesting will be an examination of the retinas of riboflavin deficient animals since this substance can be demonstrated in the normal retina (559)

Lacrimal Glands The most extensive changes occur in the ducts of the periocular glands as a result of vitamin A deficiency (312) Hyperkeratosis leads to obstruction of the ducts which results in atrophy of the glandular epithelium and absence of secretion The tarsal glands of sodium depleted rats are obstructed apparently by a caking of secretion along the lid margins (110) In rats deficient in pantothenic acid the Harderian gland secretes an excessive amount of pigment which has been demonstrated to be corproporphyrin (607) A similar porphyrin pigment is elaborated when toxic amounts of choline chloride are administered to this species (750)

Liver

Hepatic damage resulting from nutritional deficiency of one sort or another has engaged the attention of numerous investigators in recent years One must always bear in mind that some fatty infiltration accompanies partial inanition Preeminently, deficiency in choline with an attendant deficiency in methionine leads to extreme fatty alterations in the liver cells (665, 672, 673, 674 676) Such fatty infiltration may be followed by necrosis of hepatic cells and replacement by scars, that is, the production of cirrhosis Other factors which influence the resultant changes such as the fat and carbohydrate content of the diet have been discussed elsewhere (page 195) Cystine deficiency in the presence of adequate dietary choline leads to hemorrhagic necrosis of the liver From the evidence at hand it would appear that the effects of cystine and choline deficiencies are different —the former producing necrosis while in the latter fatty infiltration followed by necrosis and scarring are seen That a deficiency of either of two substances which are derived from methionine and which appear to be so closely related in metabolic processes should produce dissimilar effects on the liver is a curious fact that requires further study

In addition to these essential nutrients fatty infiltration of the liver is associated with deficiencies in others inositol in rats (686 733) pantothenic

tioned Calcium-deficient rations, however, lead to ulceration of the gastric antrum in rats (44) and to gastric lesions of dogs (36)

Intestines The only other nutritional deficiency besides that of calcium to affect the intestinal tract is an inadequacy of pantothenic acid (610) Primarily in the colon, but in the lower portion of the small intestine as well extensive alterations of the mucosa are found in swine Such changes lead to severe ulceration accompanied by copious diarrhea The intestinal flora of such animals has not been studied and obviously should be to determine if this has any role in the pathogenesis of the lesions The relation of such changes to those encountered in the blacktongue syndrome of dogs must likewise be investigated for it will be recalled the lesions are alike

Eye and Paraocular Glands

Cornea Since the description of corneal vascularization in riboflavin-deficient animals (557), this change has been observed as a result of inadequacy of several other nutrients For instance, invasion of the substantia propria occurs when dietary sodium (110) and zinc (185) are insufficient, in addition similar changes have been observed when diets deficient in the amino acids tryptophane (233) lysine (242), or histidine (250) are employed The cause for the ingrowth of capillaries is not at all clear, so that the entire problem raises several interesting possibilities as to how damage to the cornea is produced One is inclined to ascribe the capillary ingrowth to injury of the cornea itself a change similar to that which is seen when the avascular cornea is deliberately traumatized (597) Injury can, of course, result from interference with the nutrition of the cornea via the tears, blood vessels at the limbus or aqueous humor, providing contact with the oxygen of the external environment is not impaired Morphologic studies reported thus far have not stated whether damage to epithelium precedes capillary invasion Leukocytic infiltration is observed in sodium deficient rats (110) before capillaries penetrate the cornea A similar sequence occurs in vitamin A deficiency following keratinization of the corneal epithelium (557) That corneal vascularization may result from damage to the epithelium as a result of inadequate nutrients being furnished these cells by the tears has been mentioned elsewhere (page 163) More studies must be made to determine if there is any morphologic evidence of epithelial damage in other deficient states Equally important is the need for an examination of the cornea in other species since the question arises of course, as to whether this structure in the rat is unique in that it is more sensitive to a variety of nutritional deficiencies than is the cornea of other animals

Lens Cataract has been reported to result from a deficiency of calcium (467), tryptophane (232, 233), and riboflavin (561) The morphology of the different types of change in the lens has already been described The

Pancreas

Aside from the familiar lesion in the duct epithelium in vitamin A deficiency no changes have been described in the pancreas in other nutritional deficiencies This is true of the islands of Langerhans as well as the acinar tissue

Adrenal

The adrenal gland is only involved in a clear-cut way by a deficiency of one nutrient, pantothenic acid This change the pathogenesis of which is not at all clear, has only been described in one species the rat (608) Such animals exhibit "hemorrhagic necrosis" of the adrenal tissues However whether the primary site of damage is the blood vessels, the medulla, or cortical cells is not at all clear from the observations thus far published The adrenal is occasionally said to be damaged in choline-deficient rats in which a vascular lesion may be the precipitating factor (690)

Genito-Urinary Tract

Kidneys Renal lesions have been produced thus far by deficiency of any one of five nutrients potassium (87 89) magnesium (60, 64 65, 66) chlorine (788), choline (615 664 682) and linoleic acid (742) In all, lesions appear to be most extensive in the tubular portions

How much the pathogenesis of each may have in common is difficult to apprise until studies employing similar basic diets are made In potassium-deficient rats accumulations of non-doubly refractile lipoid in the tubular epithelium precede necrosis of the cells The calcification which occurs is apparently secondary to these cellular changes Whether fat accumulates in the tubular epithelial cells and precedes necrosis which is seen in choline-deficient animals is unknown and should be investigated in view of the tremendous fatty infiltration which occurs in the liver So too, the changes in magnesium-deficient animals should be reinvestigated especially the early stages so that some idea of the pathogenesis of the lesions may be obtained The damage hitherto described in fatty acid-deficient rats is difficult to interpret The most prominent change appears to be injury to the tubular epithelial cells, but here again the pathogenesis is obscure In chloride deficiency, kidney disease is apparently produced by a precipitation of calcium salts in the tubules with obstruction

Renal Pelvis, Ureter, and Bladder The epithelial lining these structures undergoes keratinizing metaplasia as a result of vitamin A deficiency No changes have been described in other deficient states

Testis The male germinal epithelium is extremely sensitive to inanition Consequently testicular atrophy is a prominent part of many nutritional deficiencies Specific and irreversible damage occurs in vitamin E deficiency

acid in dogs (612), pyridoxine in swine (652) and rats (656), and riboflavin in dogs (554) and possibly swine (556) *The histological distribution* of the fat in relation to the liver lobule has not been pointedly studied in a single species, this should be investigated So too, the morphological distribution *of fat as revealed by histochemical studies should be correlated with* the partition of total fat, fatty acids cholesterol, and phospholipids as determined by chemical analysis

Because of the great interest in dietary hepatic damage during recent years the role of nutrition in the pathogenesis of liver disease in the human has received considerable attention Some promising results have already been reported That a certain proportion of chronic alcoholics, whose diets are notoriously inadequate, will die with livers containing increased amounts of fat has been demonstrated (751) Whereas the total fat content of the normal liver averages about 5 gm percent those from a group of chronic alcoholics averaged 12 gm percent, wide variations occurred in this group of 25 subjects the greatest amount being 34 8 gm percent In a series of five cirrhotics the fat values conformed more with the normal group It is well known that the cirrhotic liver at autopsy may or may not contain appreciable quantities of fat histologically The relationship of fat accumulation in the human liver to cirrhosis has been a much discussed question, many believing that cirrhosis is the ultimate outcome of massive fatty infiltration of the liver cells in some cases at least (752) In view of the frequently observed and simultaneous occurrence of severe fatty infiltration and early fibrosis, of the curative effects of choline in the liver lesions of rats and effect of methionine on experimental liver damage in dogs it would seem desirable to pay careful attention to the nutrition of the patient with portal cirrhosis This question is being studied today in several clinics where adequate diets supplemented by yeast and liver extract are being employed There seems to be a significant difference in the clinical course between treated and untreated groups, for instance in one study (753) a larger portion of the treated patients survived one or two years following the onset of ascites than did those of the control group and at the end of the second year 45% of the former were alive while only 21% of the untreated group were living

The effects of methionine in retarding or ameliorating liver lesions in protein-depleted dogs poisoned by a variety of agents have been referred to It is unlikely that this amino acid will protect the normal animal There are several recent reports of the use of methionine and casein digests in humans poisoned by various toxic agents For instance methionine and casein digests have been used in the treatment of a patient who inadvertently ingested 30 to 40 milliliters of carbontetrachloride (754) Such treatment was successful but obviously more cases will have to be observed before definite conclusions can be drawn

replaced by keratinizing epithelium Studies of other deficiencies have failed to reveal specific alteration in these tissues

Thyroid Gland

It will be recalled that the thyroid gland is prone to exhibit morphological changes (usually hyperplasia) under a variety of stimuli It does appear, however, that iodine deficiency leads to epithelial hyperplasia (201, 202, 203, 204 205) The reason for the hyperplasia is doubtless due to increased stimulation of the thyroid epithelium by the hypophysis because of a decrease in circulating thyroid hormone It would be interesting to study the effects of iodine deficiency in the hypophysectomized animal The counterpart of colloid goiter, which in man is thought to result from iodine deficiency, has not been produced in experimental animals placed on low-iodine regimens

Parathyroid Glands

Because of its intimate role in calcium metabolism it is not surprising that a deficiency of lime salts leads to parathyroid hyperplasia (45) There is adequate evidence that a lowering of the blood calcium stimulates parathyroid secretion and in certain other metabolic disturbances such as nephritis enlargement of this gland is encountered Although it is claimed that phosphorus deficiency also produces enlargement of the parathyroids, this observation requires confirmation since others including ourselves (115) have not observed similar changes on diets deficient in phosphorus alone

Hypophysis

In reports dealing with the response of tissues to deficiency of an essential nutrient, the hypophysis is seldom mentioned, which means of course that it is not usually examined in routine studies It has been claimed that this important gland of internal secretion is twice as large as normal in the leucine-deficient rat an observation which certainly requires confirmation A secondary manifestation of vitamin A deficiency which apparently depends on increased intracranial pressure is the presence of cysts in the hypophysis of calves deficient in this vitamin

Mesenchymal Tissues

Connective Tissues Ascorbic acid is apparently of importance in determining the differentiation of mesenchymal cells into fibroblasts, so that when this vitamin is absent the potential connective tissue cell is unable to

although it will be recalled (page 122) that only the rat (407, 408) and guinea pig (409) show this change. In such animals if the deficient state is severe enough there is complete destruction of the spermatogonia, only the Sertoli cells remain. The paired-feeding technique should be utilized to study the effects of deficiencies of the other essential nutrients more carefully. This is certainly true of arginine deficiency in which there is some experimental evidence of a specific effect of this nutrient on the testis of man (255).

Accessory Male Sexual Apparatus. Characteristic changes occur in the epididymis, prostate, seminal vesicles and coagulating glands in vitamin A deficiency. When the affects of inanition are ruled out no other nutrients have as yet shown to effect these structures.

Ovary. In vitamin E deficiency the ova can be said to be involved inasmuch as the developing embryo and its tissues appear to bear the brunt of the damage incurred (402, 403, 404, 405). Depending on the degree of maternal deficiency various pathological alterations are seen in the embryo from the tenth or eleventh day on. Reproduction is of course interfered with as a result of numerous other deficiencies. However, the question of inanition has not been ruled out in any thus far reported, that is, animals which fail to gain or gain weight poorly on deficient diets have not been compared with animals on the control diet whose food intake is restricted so that the weight gain in both are comparable. This is brought out in a study of pantothenic acid deficiency on the reproductive activity of the rat in which it is concluded that the factor of inanition was eliminated in animals made deficient before and at the day of mating. However, this experiment exemplifies again the probable inaccuracies inherent in the paired-feeding technique, for the deficient animals gained an average of one fourth that of their adequately paired-fed and non-deficient controls (766). The role of vitamin A needs to be clarified since localized areas of inflammation have been noted at the fetal-maternal junction; the reason for this is not clear. Manganese deficiency may lead to specific lesions in the fetus which have their inception in utero. Histological studies have not been reported in such animals.

Accessory Female Sex Organs. The uterus, vagina and vulva of vitamin A deficient animals exhibit the characteristic keratinizing metaplasia seen elsewhere. Lesions in these tissues as a result of a deficiency of other nutrients have not been reported, at least convincing evidence of changes other than those which must be ascribed to inanition has not been brought forward.

Respiratory Tract

Trachea and Bronchi. The air passages exhibit a change in their lining epithelium in vitamin A deficiency when normal ciliated columnar cells are

replaced by keratinizing epithelium Studies of other deficiencies have failed to reveal specific alteration in these tissues

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Mesenchymal Tissues

Connective Tissues Ascorbic acid is apparently of importance in determining the differentiation of mesenchymal cells into fibroblasts so that when this vitamin is absent the potential connective tissue cell is unable to

lay down argyrophilic fibers which normally are converted into collagen (463) The exact mechanism of this process in the formation of intracellular substances is not as yet entirely clear The theory that connective tissue cells, or at least potential fibroblasts, are only able to deposit a fluid like material that jells into collagen under the influence of ascorbic acid is an attractive hypothesis but one which must be investigated further

The subject of "intercellular cement substance," that is the material which holds endothelial and other cells together, should be mentioned here also Whether this material is related to the substances which we call "collagen" is not clear at this time It is thought by some that calcium is more important than ascorbic acid in maintaining the integrity of this material (770) The whole problem is extremely complex, especially since we are so ignorant of the chemical nature of collagenous materials and 'cement substance The relationships of calcium and ascorbic acid have been reviewed (344), especially in relation to intercellular substances and cell surfaces

Cartilage and Bone Since these two specialized tissues are so closely related, they will be discussed together Bone growth, in which of course cartilage growth is involved can be impaired in several ways disturbance in the osteogenic-osteolytic balance disturbance in growth of cartilage or disturbance in the deposition of the inorganic elements in cartilagenous matrix substance and/or osteoid

Scurvy is the disease which exemplifies the first type, for in this malady there is an inability of fibroblasts to form osteoid The pathological consequences of this have been described in detail Lesions of vitamin C deficiency develop because there is no disturbance in the growth of cartilage thus ascorbic acid deficiency differs from vitamin A deficiency in that in the latter there is not only a decrease in osteoblastic activity, but also a slowing up of the growth of cartilage cells Bone changes in vitamin A deficient animals are morphologically identical with those seen in inanition but must be mentioned because the skeleton is singled out from the rest of the tissues when vitamin A is absent from the diet and the specific action of this vitamin on bone can be beautifully brought out when excessive amounts are administered (771)

Rickets furnishes an example of the third type of bone disease defective deposition of calcium and phosphorus in the cartilage and osteoid which, of course may occur from a deficiency of calcium, phosphorus or vitamin D Rickets can be produced not only by an abnormal ratio of calcium and phosphorus in the diet, but by excessively low dietary levels of these two elements (377) The cartilage growth component of rickets is not clear Whether vitamin D or a deranged calcium phosphorus ratio or level in the intercellular fluids or mechanical effects predispose to the refractoriness of the cells to destruction are subjects for further investigation

The role of manganese in bone formation is as yet not settled but since bony deformities have been noted (169, 171, 172, 183), this element may play a role in osteogenesis which is interesting in view of its relation to phosphatase activity (165)

Teeth As the dental structures are derivatives of both epithelium (enamel organ) and dentine, they may be discussed from two stand-points depending on which of these two components is initially affected The enamel organ is primarily damaged in both magnesium and vitamin A deficiencies It is unfortunate that this structure has not been studied more fully in other deficiencies in which ectodermal structures particularly the skin are severely involved In magnesium deficient animals ameloblasts atrophy with the result that the enamel is hypoplastic Secondary changes occur in the formation of dentine The enamel organ acts as an organizer of the odontoblasts and in vitamin A deficiency a lack of this organizing influence is strikingly seen Because of physiological abnormalities in the ameloblasts the odontoblasts do not differentiate with a result that the formation of dentine is irregular or absent The enamel organ in this instance seems to have the same effect on the organization of odontoblasts as does vitamin C

Interest has also been aroused in teeth because of another functional activity of the ameloblasts the formation of the familiar yellow iron containing pigment of the rat s incisor Failure of this pigment to be deposited has been noted to result from deficiency of several nutrients iron tryptophane, vitamin A, and alpha-tocopherol Since this material contains iron it is not surprising that iron deficiency leads to its disappearance (616) The relations of tryptophane to the pigment is less clear although this amino acid is of course intimately allied to hemoglobin formation (229 234) Vitamin A deficiency leads to changes in the ameloblasts which would explain the dental achromia in this deficiency Absence of the pigment in vitamin E deficient rats cannot be explained at present It is of interest that when certain elements such as cadmium and fluorine are included in the diet of rats the yellow pigment does not appear (755)

It was noted above (page 100) that the odontoblasts are organized by the enamel epithelium so that various secondary changes are seen in the dentine as a result of damage to the enamel organ Certain deficiencies, however primarily affect the physiology of dentine for instance vitamin C deprivation leads to a cessation of formation of dentine in conformity with its generalized influence on the elaboration or inter-cellular substances of which dentine is one Defects in the formation of dentine and of the tooth supporting structures are characteristic of the scorbutic state (474 475 476 477) A somewhat different situation prevails in rickets here the odontoblastic activity is not impaired but the dentine which is formed is not calcified because of the disturbance in calcium and phosphorus metabolism (380

lay down argyrophilic fibers which normally are converted into collagen (463). The exact mechanism of this process in the formation of intracellular substances is not as yet entirely clear. The theory that connective tissue cells, or at least potential fibroblasts, are only able to deposit a fluid-like material that jells into collagen under the influence of ascorbic acid is an attractive hypothesis but one which must be investigated further.

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sults from interference with the metabolism of the nucleated forms of these cells (587) Such an hypothesis requires further study

When considering the formation of hemoglobin, there are a few more data available Since glycine has been shown to be one of the precursors of

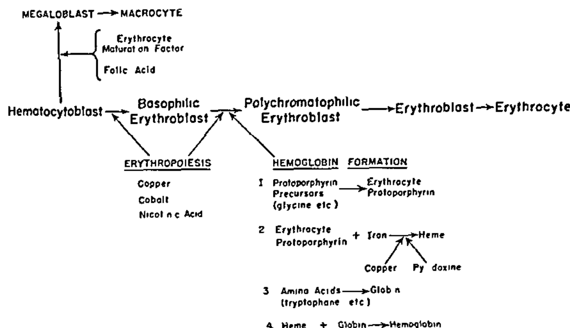


Figure 72

erythrocyte proptoporphyrin (756) it is obvious that a deficiency of the essential amino acids which are precursors of glycine, as well as any other necessary ones will have an influence on the elaboration of protoporphyrin Protoporphyrin combines with iron to form heme, an important reaction and of course, a crucial one in the development of iron-deficiency anemia That copper and pyridoxine catalize this reaction must await further proof though when these nutrients are not present in the diet iron pigment accumulates in the tissues (146, 652) and hyperferremia has been observed in pyridoxine-deficient animals (652) Although amino acids are necessary for the formation of heme they are even more important in the synthesis of globin Since this material contains most of the essential amino acids, it is apparent that a deficiency of one or more of these building stones will lead to poor production of globin, it will be recalled further from Table V that a deficiency of these essential amino acids leads to a depression of hemoglobin formation in certain species

381) Although enamel hypoplasia has not been observed on low phosphorus diets, it has been noted when the calcium intake is restricted (382)

Blood-Forming Tissues, Vessels and the Coagulation Mechanism

Red Blood Cells A notable example of the effects of deficiencies of the essential nutrients is a consideration of erythrocytes and hemoglobin formation The essential amino-acids at least 3 elements (iron copper, and cobalt) and several vitamins (pyridoxine riboflavin nicotinic acid and folic acid) have all been implicated in erythropoiesis

To gain a better perspective, a brief review of normal red blood cell and hemoglobin formation will be advantageous It will be recalled that erythrocytes are derived from a primitive stem cell which many call the hemocytoblast In the maturation of this cell a series of increasing adult forms is encountered basophilic erythroblast →polychromatophilic erythroblast →normoblast or erythroblast →normocyte or erythrocyte Hemoglobin *which appears somewhere at the end of the basophilic erythroblast stage,* is an oxygen carrying pigment derived from protoporphyrin whose precursors are not all known though one appears to be glycine (756) At any rate, erythrocyte protoporphyrin combines with iron to form heme In the meantime the globin molecule is being synthesized from amino acids Finally, heme and globin are joined to form hemoglobin Whether hemoglobin is formed in whole or in part by the developing erythrocyte is not known We are thus faced with the task of attempting to determine just where the various essential nutrients fit into the process of erythropoiesis and hemoglobin synthesis Figure 72 has been devised in an attempt to effect this understanding fully realizing that such a diagram must contain many errors, since species differences are so very important and our knowledge is so inadequate

Included in the figure is an offshoot, the megaloblast and its successors The place of this cell in blood formation is extremely controversial For the present discussion we shall look upon the megaloblast as an abnormal cell fully realizing that many feel it is a normal component of the bone marrow

Three nutrients appear to stimulate erythropoiesis *per se* *Copper* is said to promote red blood cell formation even in the absence of hemoglobin production (141) The well known stimulation of erythropoiesis by *cobalt* makes it likely that, when this element is not present in the diet, there will be a depression of red blood cell formation (620) Since *nicotinic acid* is necessary for the formation of the phosphopyridine nucleotides it has been suggested that the anemia which develops in nicotinic acid deficient dogs re-

Table VIII

BLOOD AND TISSUE CHANGES IN NUTRITIONAL ANEMIAS

DEFICIENT NUTRIENT		BONE MARROW HYPERPLASIA	HEMOSIDEROSIS			HYPERFERREMIA
			SPLEEN	LIVER	BONE MARROW	
IRON	Microcytic hypochromic	?	0	0	0	0
PYRIDOXINE	Microcytic hypochromic	+	+	+	+	+
COPPER	Microcytic hypochromic	?	+	+	+	?
TRYPTOPHANE	Normocytic normochromic	0	0	0	0	0
FOLIC ACID	Macrocytic hyperchromic	?	?	?	?	?
NICOTINIC ACID	Macrocytic or normocytic	?	+	?	?	?
RIBOFLAVIN	?*	0	0	0	0	?
COBALT	?	?	+	+	+	?

*No uniformity

Early in the history of the disease, pernicious anemia, large cells were noted in the bone marrow and were called megaloblasts. Today, some hematologists consider these cells to be abnormal forms, while others regard them as normal components of the bone marrow and a stage in the development of all erythrocytes. They are seen in largest numbers when as essential material the erythrocyte maturing factor (EMF) is lacking. This factor is formed normally by the union of an extrinsic dietary substance, as yet unidentified, and an intrinsic factor elaborated by the gastric mucosa which has also not been identified or synthesized. Pernicious anemia can be looked upon as a conditioned deficiency resulting from a diseased stomach which is unable to elaborate the intrinsic factor. Folic acid causes the megaloblastic bone marrow of pernicious anemia and other macrocytic anemias to revert to a normoblastic type; this essential nutrient, therefore, appears to be related to the erythrocyte maturing factor. At the present writing, however, the relationship of folic acid to the extrinsic, the intrinsic and the final product, the EMF, must await further investigation.

Anemia has been described in riboflavin deficient animals—rats (712), swine (555), and dogs (554, 564); the place of this vitamin in hematopoiesis is obscure, however, since the anemia is not well enough characterized. Table VIII *summarizes some of the characteristics of the various anemias which* have been discussed. Many species containing question marks remain to be filled in.

White Blood Cells. Much less attention has been directed at the study of disturbances in white blood cell formation than those of the red cell series. Nutritional agranulocytosis can be cured by the administration of folic acid (719); the only other instance of a disturbance in white blood cell formation results from deficiency of nicotinic acid in dogs (587).

Blood Vessels. From studies of healing wounds in absolute scorbutus it is concluded that although endothelial cells are able to proliferate, capillaries cannot form when ascorbic acid is not present (463). This question needs further study since, for instance, there is no retardation of capillary invasion in bones placed in casts to prevent the mechanical manifestations of stress and strain (472). The question of increased capillary fragility in scurvy also needs more careful investigation. So too, whether the hemorrhages which occur in calcium (36, 42) and vitamin K (443, 444) deficiencies are the results of poor clotting alone or whether there is actual damage to capillary endothelium, also requires pointed study.

Inasmuch as no mention has been made of so-called vitamin P or citrin this is an appropriate place to discuss this material, a flavonal which was first described by Szent-Györgyi and his associates in 1936 (598). The material was extracted from citrus fruits and differed from ascorbic acid. It was postulated that it influenced the permeability of capillaries. During

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TRYPTOPHANE	Normocytic normochromic	0	0	0	0	0
FOLIC ACID	Macrocytic hyperchromic	?	?	?	?	?
NICOTINIC ACID	Macrocytic or normocytic	?	+	?	?	?
RIBOFLAVIN	?*	0	0	0	0	?
COBALT	?	?	+	+	+	?

*No uniformity

the following years there was confusion over the exact relationship of vitamin P to the scorbutic state However, some reports seem to indicate that vitamin P is important in maintaining the integrity of capillaries even when ascorbic acid is present (786, 789) At the present time it is wise to withhold judgment awaiting further investigations

Blood Clotting Mechanism Nutritional deficiencies play an important role in disturbances of the coagulation of blood since two very important factors in blood-clotting are affected prothrombin and calcium The first of course, is dependent on an adequate ingestion and absorption of vitamin K and has been discussed elsewhere (page 128) *Hemorrhage is also a manifestation* of experimental calcium deficiency (36, 42) since calcium ions are necessary for the reaction prothrombin →thrombin A deficiency in fibrinogen can conceivably occur as the result of lack of dietary amino acids though no specific experimental evidence is available on this point

Muscle Tissues

Cardiac Muscle Necrosis of myocardial fibers has been found to result from both potassium (page 33) and thiamine (page 146) deficiencies *In* either case, individual or groups of muscle fibers lose their striations and become hyaline and necrotic There is a varying degree of cellular infiltration If the animal survives long enough, connective tissue scars replace the areas where muscle fibers have been destroyed In potassium deficiency the lesions tend to predominate in the ventricular musculature, little change has been noted in the auricles In contrast, the latter tissues of thiamine-deficient animals seem to be affected before the ventricles but later the walls of all chambers are severely involved It is of interest that in rats at least, when potassium and thiamine deficiencies are produced simultaneously, no myocardial necroses develop It is of some interest though the significance is not at all clear, that when large amounts of ethyl laurate (35-40 percent) are incorporated in a choline deficient diet myocardial necroses similar to those encountered *in potassium or thiamine-deficient* animals are found in rats (779) A combination of these might yield interesting results

Rats placed on vitamin E rations for prolonged periods exhibit extensive scarring of the myocardium (425) Apparently there is first a deposition of ceroid in the myocardial fibers followed by necroses Connective tissue is found about the bundles of muscle fibers and imbedded in the collagen are macrophages filled with this acid-fast pigment Dr Karl E Mason has kindly allowed us to examine his material *The histological picture is strikingly* different from that which is seen when potassium or thiamine deficiencies are present The chronicity and presence of ceroid are doubtless only two of

many factors which will be found to account for this Deficiencies of certain other nutrients are said to lead to heart damage Ascorbic acid deficiency in guinea pigs may produce focal lesions in the heart (478), then too cases of sudden death have been observed in children who exhibit severe manifestations of scurvy in the skeleton (479)

Striated Muscle Extensive changes occur in the skeletal muscles as a result of deficiency of alpha tocopherol Such changes which have been observed in several species (page 124) are characterized by hyaline degeneration, fatty infiltration necrosis, edema and sometimes calcification Lesions in the skeletal musculature as a result of other deficiencies require further investigation Dystrophic lesions have been produced by simultaneous deficiency of potassium and thiamine although the muscle fibers are unaffected when either of these nutrients is absent alone (96)

Studies of nutritional muscular dystrophy are of course, of importance because the lesions are identical with those which are seen in many instances of human disease A further problem of great interest is the relationship of the development of dystrophic lesions to nerve supply It will be recalled that when the nerves to a leg are cut morphological change in the muscle fail to appear when the animal is placed on a vitamin E-deficient diet It would be interesting to compare the respiration of such muscle *in vitro* with that from the opposite side and normal controls Whether motor sensory or sympathetic fibers are responsible is, of course another intriguing question

Involuntary Muscle In Mammalia no lesions have been described as yet in the smooth muscle of animals deficient in one or more of the essential nutrients Pigment, of course occurs in smooth muscle of vitamin E-deficient animals (425 428) but the significance of this deposit is not clear at this time

Nervous Tissues

Peripheral Nerves Lesions consisting of myelin degeneration have been noted in the peripheral nerves in a number of deficient states However many of these reports indicate that the diets employed have been lacking in several essential nutrients in addition whether the site of the lesions is in the motor or sensory fibers has in most instances not been determined A fairly accurate estimation of which type of nerve is affected can be obtained by examining the cells of the anterior horns and dorsal ganglia as well as the ventral and dorsal roots Specific alterations of the sensory neuron have been produced in swine on diets deficient in pantothenic and/or pyridoxine (611) The motor neuron is not involved by a deficiency of either of these two nutrients since degeneration of the ventral roots and anterior horn

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Central Nervous System Before commenting on the morphological lesions which may be produced in the brain when animals are placed on deficient diets certain well defined manifestations of physiological disturbance must be mentioned In several species a peculiar syndrome of hyper-excitability followed by tonic convulsions has been produced by deficiencies of either magnesium (54, 55) or pyridoxine (654, 655) In swine deficient in the latter nutrient and exhibiting such signs no pathological alterations have been detected in the brain This is to be expected since such animals may spontaneously recover from an attack or may be cured by the administration of appropriate amounts of the missing nutrient It is of interest that similar attacks have been observed in rats on a potassium deficient diet which had been supplemented with either rubidium or cesium in order to replace the missing essential nutrient (94) Inasmuch as all of these peculiar convulsions seem to involve the muscular system as well as the nervous tissues the possibility that the former may also play a role must not be lost sight of Until pointed physiological studies are carried out the exact locale or locales responsible for these syndromes must be held in abeyance It would be most interesting to observe the electroencephalographic pattern of animals exhibiting such seizures

Perhaps the most extensive morphological lesions which have been reported to result from deficiency of a single nutrient are those which occur in the brain when dietary copper is inadequate (144 145) Virtually complete destruction of the white matter has been found in the tissues of newborn lambs whose mothers apparently had insufficient dietary copper The lesions are of great interest to the neuropathologist since they closely resemble cases of progressive symmetrical demyelinization which are observed in man Morphological changes in the brain as a result of other deficiencies are not at all clear cut, thiamine deficiency leads to physiological disturbances and these, as well as morphological alterations have been described elsewhere (page 152)

Cerebral hemorrhage resulting from low prothrombin levels has been noted in vitamin K-deficient animals and a similar state of affairs may be responsible for the hemorrhages which have been noted in choline-deficient rats In this instance one must postulate that the liver is damaged (there is of course, morphological and biochemical evidence that it is) and is unable to form adequate amounts of prothrombin The hemorrhages which are said to occur in calcium deficient animals should be further studied

Vitamin A deficiency provides an excellent technique with which to study the effects of increased intracranial pressure since it will be recalled that the brain of young animals deficient in this nutrient continues to grow while its bony covering stops increasing in size The effects of pressure on

cells is not observed. In pyridoxine-deficient animals the initial site of injury is in the peripheral nerves where myelin degeneration appears, such changes being noted before degenerative phenomenon appear in the ganglion cells of the dorsal roots. As the deficiency progresses the dorsal root fibers and ascending tracts of the spinal cord are involved but during the course of the disease chromotolysis is not observed in the cell body although atrophy and necrosis occur. In contrast the initial change in pantothenic acid deficient swine is dissolution of the Nissl substance, a phenomenon which precedes any morphologic evidence of degeneration of myelin and axoplasm of the peripheral or central portions of the nerve. As time goes on changes in these structures similar to those seen initially in pyridoxine deficiency appear, and, in addition, there is degeneration of the dorsal root fibers and the ascending tracts of the spinal cord. Thus there appear to be two different patterns in the pathogenesis of the changes in the sensory neuron in these deficient states, and because of this difference the hypothesis has been presented that pyridoxine is intimately connected with the metabolism of myelin and that pantothenic acid affects the integrity of the cell body itself. Such an hypothesis based on morphological evidence alone requires further physiological confirmation. The subject is of great theoretical interest however, in view of the two types of neurological degeneration which one encounters in the nervous tissues of man, that is myelinoclastic disease such as multiple sclerosis in which the myelin is primarily affected and polioclastic disease such as epidemic encephalitis in which the cell body is first injured and myelin degeneration is secondary. The effects of these two deficiencies on the nervous tissues of other species should be studied in order to confirm or reject the observations which have been reported in swine.

As was noted above data on changes in the peripheral nervous tissues in other deficient states are very inadequate. Degeneration of the sensory neuron has been observed in a few swine placed on a diet low in protein content and deficient in nicotinic acid as well (592). So too myelin degeneration has been observed in representatives of several species deficient in riboflavin (553, 555, 556, 563). In such animals, the site of lesions whether in the sensory or motor fibers has not been determined as yet. It will be recalled that in Mammalia there is no good evidence that thiamine deficiency alone leads to degeneration of the peripheral nerves, the question of its relationship to the integrity of this portion of the nervous system requires further study in birds.

From the investigations already reported it is obvious that the metabolism of motor and sensory neurons is different. It would therefore seem desirable to study the respiration of these neurons *in vitro* and to determine the response of cells from deficient animals to the addition of certain specific nutrients already known to effect them.

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neurons and the development of papilledema would seem two subjects which could be investigated by this means

Deficiency disease therefore affords the neuropathologist a potent tool with which to study some of the fundamental reactions to injury in the central nervous system and it is to be hoped that such techniques will be applied in the future It may, of course, be added that competent neuro histologists can doubtless add a great deal to the studies already reported and should also find the investigation of other deficiency states of interest

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SUBJECT INDEX

THIS BOOK

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By RICHARD H FOLLIS, JR., M D

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